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RESPIRATION OF THE EGG AND YOUNG OF THE SMOOTH GREEN SNAKE, LIOPELTIS VERNALIS (HARLAN)

By

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(*Received January 22, 1937*)

Observations on the metabolism of the reptilian egg are rarely encountered in the literature. Yet in 1847 Baudrimont and Martin de St Ange reached the important conclusion that the CO_2 evolved from snake eggs was the result of a true combustion. This was held to account for the heat production of the eggs of the python during incubation which had been reported by Valenciennes (1841). Bohr's ('04) study of respiration of the egg of *Coluber natrix* showed a definite decline in respiratory intensity with age. He gives evidence for the conclusion that there is a dominant, complete combustion of carbohydrate material. In the course of his extensive study of the metabolism of reptiles, Benedict ('32) became convinced that a real rise in temperature was developed by the female python during incubation. This fact is of considerable interest from the standpoint of the problem of the evolution of homoiothermism.

The effect of temperature on the respiration of the vertebrate egg and the newly hatched young seems never to have been systematically studied. Sauropsidan eggs are particularly well adapted for such analyses, especially those of reptiles which are persistently poikilothermous. It was with this objective in mind that the present study was undertaken.

MATERIALS

A clutch of eight eggs of the smooth green snake, *Liopeltis vernalis* (Harlan), was found under a decaying log close to the margin of a quiet pond near Jefferson, Mass., on August 9th 1936. One egg was injured in transportation. Another (S1) was used for purposes of identification. Four individuals (S2, S3, S4, S5) were used for respiration studies. One egg (S6) was exposed to similar conditions of experimental temperature change but was not actually in a respirometer. All eggs were kept in separate containers on moist peat moss when not under actual study.

Respiration measurements of the eggs were made at various temperatures from the second to the seventh day after being removed to the laboratory (Aug. 11 to 16). Evidences of hatching were first noted on the ninth day. By 11 A.M. on August twentieth all five snakes had emerged from the eggs. Animal S6 was the last to hatch. Respiration studies were made on the snakes on the 10th, 11th and 12th day after collecting the eggs. Attempts were made to feed the snakes with soft bodied insects and small spiders but these did not prove very successful. They moved actively while in the respirometers and none lived beyond the twentieth day.

TABLE 1
CHANGES IN WEIGHT, VOLUME AND DENSITY

	Date	S2			S3		
		Wt.	Vol.	Density	Wt.	Vol.	Density
Eggs	Aug. 11	2.292	2.1	1.091	1.938	1.7	1.140
Snakes	Aug. 22	0.990	0.95	1.042	0.886	0.8	1.108
Snakes	Aug. 29	0.908	0.9	1.009	0.796	0.75	1.061
% change	Aug. 11–22	−56.8	−54.8		−54.3	−41.2	
% change	Aug. 22–29	−8.2	−5.3		−10.2	−6.3	
		S4			S5		
Eggs	Aug. 11	1.968	1.8	1.093	2.130	2.2	0.968
Snakes	Aug. 22	0.947	0.9	1.052	0.828	0.8	1.035
Snakes	Aug. 29	0.857	0.8	1.071	0.756	0.7	1.080
% change	Aug. 11–22	−51.9	−50.0		−61.1	−63.6	
% change	Aug. 22–29	−8.8	11.1		8.7	12.5	

METHODS AND OBSERVATIONS

A modified Warburg respirometer was used to measure simultaneously the O_2 consumption and the CO_2 production at temperatures ranging from 12° to 30° C. Observations were made on both ascending and descending series of temperatures. One-half cc. of water was added to each vessel and no shaking

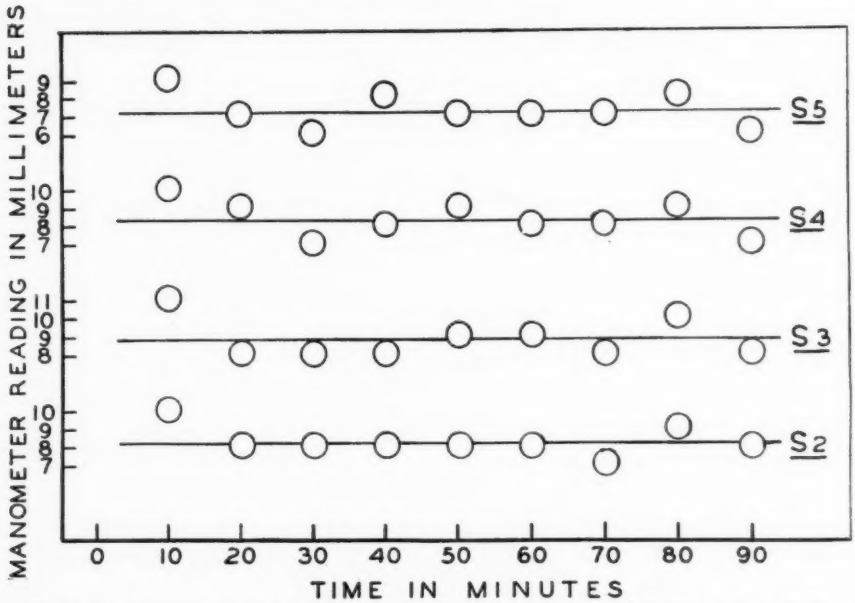

FIG. 1. Direct manometer readings for O_2 consumption of four eggs at 20.4° C. The mean value for each is represented by a line drawn through the data.

was used. A minimum of three-quarters of an hour was allowed for thermal adaptation before each run. The volume of the eggs and of the snakes was

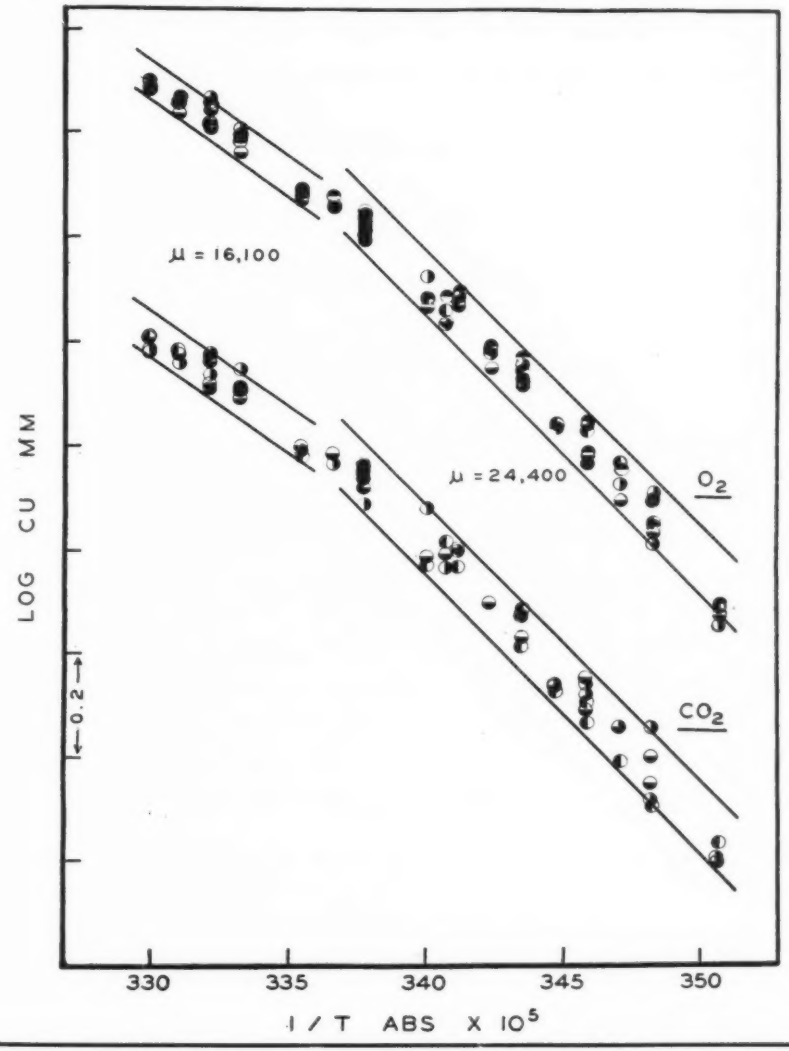

FIG. 2. Composite plot of the egg respiration of the smooth green snake, *Liopeltis vernalis*. The various symbols represent different individuals.

determined by the method of displacement using a narrow-walled 10 cc. graduate cylinder, Data concerning changes in weight, volume and density have been assembled in Table 1.

Ten-minute direct manometer readings for oxygen consumption at 20.4° C (Figure 1) showed a relatively regular rate which indicated that embryonic

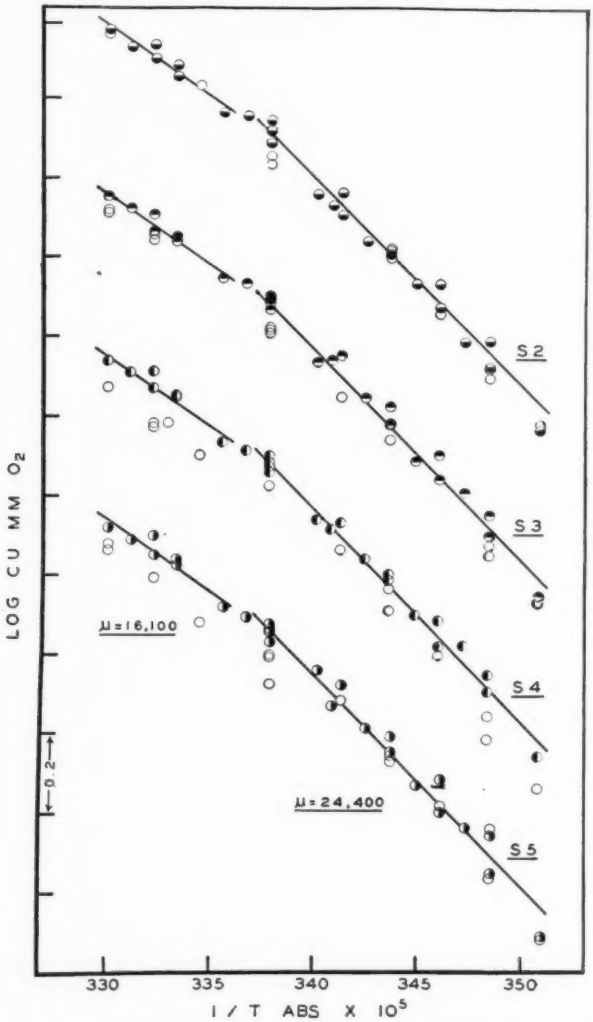

FIG. 3. Oxygen consumption of the egg and young of the smooth green snake, *Liopeltis vernalis*. Various symbols have been used for different eggs. Open circles represent the respiration of the corresponding hatched snakes.

movements were not sufficient to effect the accuracy of the hourly values used in the temperature plots.

All data obtained for O_2 consumption and CO_2 production were plotted according to the Arrhenius equation (cf., Crozier '24, Crozier & Hoagland '34, Needham '31). Individual plots were arranged in such a way as to superimpose the data obtained from the four eggs studied (Figure 2). A temperature characteristic (μ) of 24,400± was obtained for respiration at temperatures ranging from 12° C to approximately 24° C. Exact determination for the value of the μ for temperatures between 25° to 30° C was rendered difficult due to the shortness of the range and the relatively small number of readings obtained. A value of 16,100± seemed to best describe that portion of the curve. The "break" be-

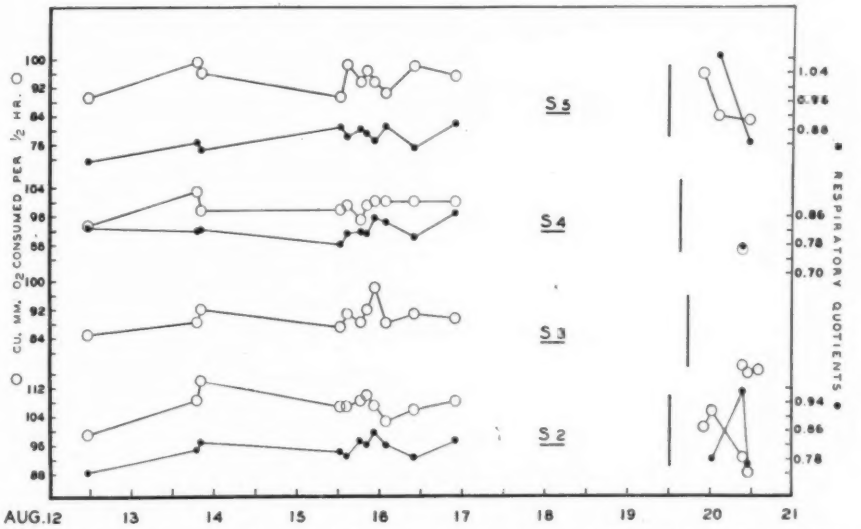

FIG. 4. Oxygen consumption (open circles) and respiratory quotients (solid circles) for the egg (left) and hatched young (right) of four green snakes. Verticle bars indicate the time of emergence.

tween the two temperature slopes appeared to be between 23° and 25° C. The results obtained for O_2 and CO_2 are essentially similar.

Individual plots are shown in Figure 3. Various symbols have been used for different eggs. Open circles represent data collected from the corresponding hatched animals. The relative respiratory rate for egg and young are fairly well correlated. The absolute respiratory rate is somewhat less for animals S4 and S5 at the high temperatures but it is notable that while there is only a slight difference between the rate of gas exhange of the egg and the hatched young, the weight and volume of the latter are more than 50 per cent less No attempt was made to determine the amount of inert material of the eggs nor the weight of the embryonic membranes. It was concluded that the values for the respiring portion of the eggs and snakes were of the same order of magnitude.

The largest number of observations were made at 23° C. Data collected on various days are presented in Figure 4 in which the O_2 consumption (open

TABLE 2

RESPIRATORY QUOTIENTS FOR EGGS AND SNAKES

Eggs	S2	S4	S5	Temp.
Aug. 11	0.7971	0.8854	0.8790	20.4
	0.7181	0.8846	0.9290	18.0
	0.8342	0.7787	0.9347	16.0
	0.5939	0.5406	0.8334	14.0
Aug. 12	0.8698	0.8455	0.9342	20.0
	0.7919	0.6564	0.8786	15.0
	0.9238	0.7510	0.8335	16.5
	0.8658	0.7702	0.9253	21.0
	0.7457	0.8297	0.8955	23.0
	0.7563	0.7873	0.8235	25.0
	0.8450	0.8864	0.9160	27.0
	0.9560	0.8325	0.8167	29.0
	0.8258	0.8093	0.8303	28.0
Aug. 13	0.8114	0.8021	0.8249	24.0
	0.8903	0.7986	0.8456	28.0
	0.8210	0.8291	0.8001	30.0
	0.8128	0.8055	0.8217	27.0
	0.8094	0.8203	0.8461	23.0
	0.8318	0.8235	0.8267	23.0
	0.8279	0.7660	0.8453	20.0
Aug. 14	0.7595	0.7420	0.8042	16.0
	0.7926	0.6358	0.9229	14.0
	0.8339	0.7269	0.8516	18.0
Aug. 15	0.8067	0.7817	0.8909	23.0
	0.7935	0.8124	0.8604	23.0
	0.8355	0.8180	0.8814	23.0
	0.8249	0.8124	0.8706	23.0
	0.8596	0.8559	0.8500	23.0
Aug. 16	0.8258	0.8424	0.8947	23.0
	0.7906	0.8016	0.8291	23.0
	0.8355	0.8696	0.8983	23.0
Average	0.8157	0.7936	0.8643	
Snakes				
Aug. 19	0.7807	0.7759		23.0
	0.9699			23.0
Aug. 20	0.7674		1.0889	23.0
			0.8479	23.0
	0.9107	0.8719	1.0889	18.0
	0.9045	0.9541	0.8479	18.0
	0.9042	0.8335	1.0212	14.0
Aug. 21	0.9680	0.8061	1.2297	14.0
	0.8685	0.9794	1.1737	12.0
	0.9001	0.8012	1.0228	16.0
	0.8159	0.7596	0.8774	20.0
			0.9099	30.0
			0.8690	30.0
			0.9476	28.0
	0.8597			26.0
Average	0.8772	0.8477	0.9854	

circles) and corresponding respiratory quotients (solid circles) are plotted against time. Verticle bars indicate the time when the snakes were seen to emerge from the eggs. No significant change in O_2 uptake by the egg seems indicated. The first evidence of shell perforation was noted about 40 hours after the last respiratory measurement. The snakes did not leave the shells until some twenty hours later.

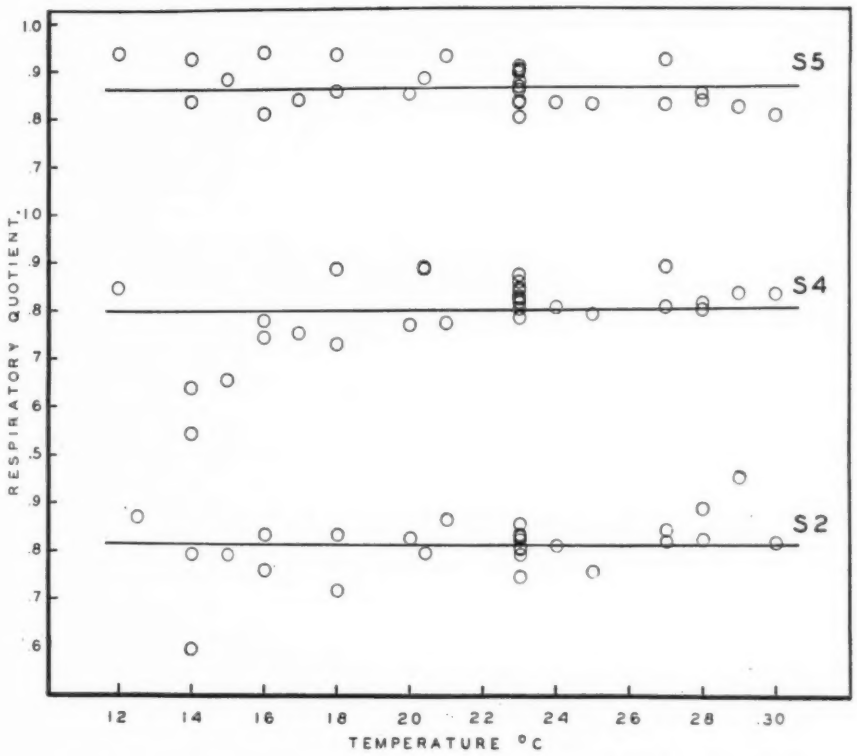

FIG. 5. Respiratory quotients as a function of temperature for the egg of the green snake.

Graphical representation of the relation between the respiratory quotients and the temperature (Figure 5) shows no significant change during the late stages of embryonic development. No shift in R Q seems indicated with the approach of hatching (cf., Table 2).

The respiratory quotient measurements for all data were found to shift from 0.8235 ± 0.0047 for the eggs to 0.9105 ± 0.0137 for the snakes. This difference being six times the probable error of the difference (Table 3) is certainly significant.

TABLE 3. COMPARISON OF MEAN RQ VALUES FOR EGGS AND SNAKES

	Mean	St. Dev.
Eggs	0.8235 ± 0.0047*	0.0675 ± 0.0033
Snakes	0.9105 ± 0.0137	0.1130 ± 0.0097
Difference	0.0870 ± 0.0145	$\frac{\text{Diff}}{\text{PE(diff)}} = 6+$

* P.E.

SUMMARY AND CONCLUSION

(1) By means of a modified Warburg respirometer the gas exchange of four eggs and corresponding hatched young of the smooth green snake, *Liopeltis vernalis* (Harlan), was measured at temperatures ranging from 12° to 30° C.

(2) Arrhenius plots of the data yielded temperature characteristics of 24,400± for temperatures ranging from 12° to 24° C and of 16,100± for temperatures of 25° to 30° C. These values were essentially similar for oxygen consumption and carbon dioxide production of both the eggs and the hatched young.

(3) The absolute rate of gas exchange of the eggs and young was of the same order of magnitude.

(4) Observations up to within 40 hours of shell perforation showed no significant shift in respiratory quotient or in the absolute rate of respiration.

(5) While the respiratory quotients remained at approximately the same level at various temperatures during the entire time of study of the egg, a statistically significant change occurred from a mean value of 0.8235 ± 0.0047 for the eggs to 0.9105 ± 0.0137 for the snakes.

(6) With the exception of the rise in *R Q* found for the snakes the results of this study lend support to the view that the act of hatching *per se* does not materially affect the fundamental processes of metabolism which are in progress.

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d-LYSINE AND GROWTH

By

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The Research Institute of The Lankenau Hospital, Philadelphia, Aided by a Grant from The International Cancer Research Foundation

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That lysine must be present in the diet of vertebrates for them to show increase in size and weight had long been known. What has not been known is the phase or process—of the many which may contribute to such increase—to which lysine is primary. These studies bring some light into this obscurity.

As usual the colonial-living form of the marine hydroid *Obelia geniculata* was the test animal. This and the experimental procedure have been described in earlier reports. References are given in another paper in This Journal (Hammett, '37).

Like numbers of test and control animals of like distribution according to state of development at the beginning of observation were simultaneously grown in fresh sea-water cultures under like conditions of temperature, illumination, and pH. Four changes into fresh solutions were made during the 24 hour course of experiment.

Stock solutions containing 0.11 gram freshly recrystallized pure d-lysine

TABLE 1

THE NUMBER OF EXPERIMENTS, COLONIES, HYDRANTHS, AND GONOPHORES USED IN THE D-LYSINE STUDIES TOGETHER WITH THE PERCENTAGE REGRESSION AND CATABOLISM EXHIBITED BY TESTS AND CONTROLS AT THE STATED CONCENTRATIONS

M / 1000×	Year	# Expt	# Colonies		# Hydranths		# Gonophors		% Regressn		% Catabolism	
			Cont	Test	Cont	Test	Cont	Test	Cont	Test	Cont	Test
1000	1936	10	150	150	2615	2606	61	104	34	68	86	83
400	1934	4	60	60	994	992	79	83	30	33	93	90
400	1936	10	150	150	2574	2573	117	128	37	47	86	82
200	1934	4	60	60	1006	1008	73	68	27	39	91	84
200	1936	10	150	150	2557	2563	126	97	42	47	84	83
100	1934	4	61	60	1051	1054	83	72	21	26	82	81
100	1936	10	150	150	2608	2606	56	73	23	28	80	83
50	1933	1	12	12	153	156	7	12	39	50	86	94
50	1934	4	60	60	1009	1015	74	101	29	47	81	73
50	1936	10	150	150	2562	2557	113	98	34	43	85	81
25	1933	4	55	55	733	725	40	44	37	42	80	84
25	1934	4	60	60	1020	1014	120	122	35	52	91	86
25	1936	10	150	150	2612	2614	101	110	41	49	75	77
16.7	1933	4	58	58	725	733	59	65	45	56	90	91
12.5	1936	10	150	150	2555	2570	72	61	42	51	81	81
10.0	1933	4	55	55	755	750	79	73	36	43	94	90
6.25	1936	5	75	75	1256	1256	59	61	29	42	54	50
Totals	—	108	1606	1605	26785	26792	1319	1372	—	—	—	—

hydrochloride in 100 cc. fresh sea-water provided daily the aliquots from which test culture solutions were made. Stock solutions were brought to the pH of the solvent by addition of appropriate N/10 NaOH.

One hundred eight experiments over a concentration range from M/1,000,000 to M/6,250 were run during three different seasons with three different sets of workers using over 50,000 animals. The repetition seemed necessary for despite the general belief lysine is indispensable to growth, no open evidence of growth reaction to the compound was at any time given in the Obelia experiments.

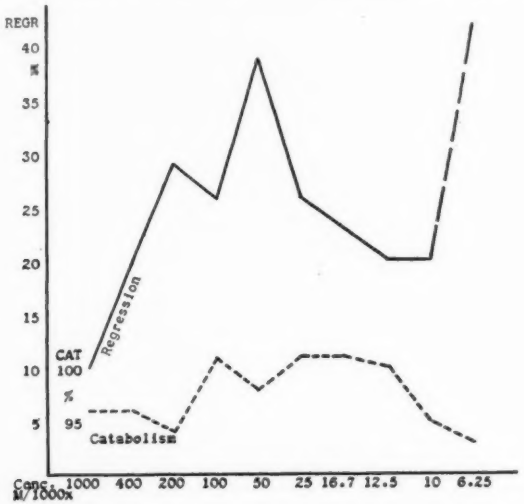

CHART 1. Effect of increasing d-lysine concentration on Obelia hydranth regression and catabolism.

Table 1 gives the raw data according to concentration and year together with the regression of the complete hydranths and the distintegration of the senile in terms of absolute percentage. Table 2 gives the direction of test deviation from control for each of the stated developmental and metabolic activities in each concentration and season. It is derived, constructed, and analyzed, as described in detail in the l-aspartic acid report (Hammett, '37).

RESULTS

The outstanding reaction was an increased regression at the amazing low concentration of M/1,000,000. No other amino acid has produced a like effect.

The course of the reaction with increasing concentration also differed. Though the shape of the curve of the combined data of the three seasons—Chart 1—has elements of likeness to those of the other amino acids (Hammett, 36, a, b, c) it differs therefrom in two significant respects. It shows no unbroken rise. The incidence and continuance of its deline are unrelated to toxicity as expressed by consistent growth retardation.

While it may be that a specific dynamic action participates in the response, something else is exhibited since the course of reaction is definitely different. This holds for both the combined and the separate data by season.

The difference justifies the assumption that d-lysine is implicated distinctively if not specifically in a different phase of metabolism than are the other amino acids.

The reaction of "growth-as-a-whole" to d-lysine concentration is separable into three phases. A phase of tendency to lesser growth in the low M/1,000,000 –M/200,000 range—a phase where test growth was the same as control as in the

TABLE 2

TREND OF TEST DEVIATION FROM CONTROL WITH RESPECT TO DEVELOPMENTAL AND METABOLIC ACTIVITY IN D-LYSINE CULTURES

M / 1000×	Year	Init. +	Init. −	Init. =	Prol. +	Prol. −	Prol. =	Diff. +	Diff. −	Diff. =	Organ. +	Organ. −	Organ. =	Main. +	Main. −	Main. =	Catab. +	Catab. −	Catab. =
1000	1936	0	3	4	3	3	2	3	4	3	0	4	5	3	7	0	3	7	0
400	1934	2	2	3	2	3	3	3	4	3	2	1	6	1	3	0	0	3	0
400	1936	3	3	1	1	4	3	2	4	4	2	3	4	1	10	0	2	7	1
200	1934	1	4	2	3	3	2	2	4	4	5	1	3	0	4	0	0	4	0
200	1936	2	2	3	3	1	4	3	2	5	1	4	4	2	7	1	4	5	1
100	1934	4	2	1	3	4	1	4	2	4	0	2	6	1	3	0	2	2	0
100	1936	1	4	2	3	1	3	1	4	5	2	2	5	0	8	2	6	3	1
50	1933	2	3	1	1	0	4	2	2	2	0	1	4	0	1	0	1	0	0
50	1934	3	1	3	3	3	2	0	3	5	2	0	6	0	4	0	1	3	0
50	1936	3	2	2	4	1	3	1	5	4	2	2	5	0	10	0	4	5	1
25	1933	0	5	2	1	4	3	2	5	3	3	0	4	1	2	1	4	0	0
25	1934	3	3	1	4	1	3	3	4	3	3	0	6	0	4	0	1	3	0
25	1936	2	3	2	3	1	4	5	4	1	1	3	5	0	10	0	6	4	0
16.7	1933	4	1	2	4	2	2	3	3	4	2	1	5	0	4	0	3	1	0
12.5	1936	1	3	3	2	4	2	1	4	4	1	3	3	1	9	0	5	4	1
10.0	1933	1	3	3	2	4	2	2	4	4	0	2	4	1	3	0	2	2	0
6.25	1936	1	4	2	1	2	5	2	2	4	2	1	3	0	5	0	2	3	0

M/200,000–M/16,700 range—and a phase of toxic retardation in concentrations of M/12,500 and over.

Thus—Table 2 shows that of the 136 developmental expressions at the low range, 37 per cent were less in tests than in controls and but 25 per cent were greater: that of the 320 expressions in the middle range, 93 were less and 93 were greater: and that of the 91 expressions in the high range, test progression was less in 40 per cent and greater in but 18.

The inhibition at low concentration—followed by no inhibition or relative or absolute growth enhancement at higher—with recurrence of inhibition at the highest has been observed with some other amino acids. Its counterpart has been recorded for bacterial growth (Wyon and McLleod, '23). Though the phenomenon is as yet inexplicable its occurrence is suggestive.

The curve of catabolic expression on Chart 1 shows this activity tends to be less than control when growth was less and the same or a bit greater when growth was not retarded.

Although "growth-as-a-whole" was unaffected by d-lysine in the non-toxic range, differential analysis brings out that proliferation expression tended to be specifically enhanced. Thus—of the 76 expressions of this activity, 38 per cent were greater in test than in control material, while but 24 per cent were less. On the other hand—of the 69 expressions of initiation, 35 per cent were greater and 38 per cent were less: of the 94 expressions of differentiation but 26 per cent were greater, while 36 per cent were less: and of the 81 expressions of organization, 20 per cent were greater, and 19 per cent were less.

Like specificity is exhibited when analysis is made on the basis of the ten sets of experiments done in the effective range. Thus—proliferation tended to be greater than control in seven and less in but two: while initiation tended to be greater in four and less in four: differentiation tended to be greater in three and less in five: and organization to be greater in four and less in four.

The same holds true when the data are combined according to single opportunity for expression of developmental potentiality. Thus—of the eight possible types of proliferation expression, seven were greater in test than control and but one was less: while of the seven potentialities for initiation, three were greater and three were less: of the ten for differentiation but two were expressed to greater degree while five were expressed to lesser: and of the nine potentialities for organization, two were expressed to greater and two to lesser degree.

This enhancement also holds, either absolutely or relatively, when the data are taken according to year or to concentration. It appears consistently regardless of how the data are grouped.

Contrariwise neither initiation nor organization show by any method of data arrangement any evidence of reaction to lysine.

Differentiation on the other hand gives, with one exception, as consistent evidence of retardation as proliferation gave of increase. Gonophore expression of differentiation activity may have been enhanced. No other single growth progression showed increase of sufficient consistency to warrant comment.

DISCUSSION

These data give no evidence that Obelia "growth-as-a-whole" is favored by d-lysine. Yet the results of work with higher animals establish the compound as indispensable to growth.*

The two sets of observations are complementary rather than contradictory when what is carried in the term "growth" is clearly set forth.

Now the measure of influence in rodent work is weight change, and the measure of influence in Obelia work is developmental change. No measurements of development were made on the rats, and no measurements of size or weight were made on obelia. Indeed the rat studies could not show whether lysine influenced developmental growth or not.

* Osborne and Mendel, '14, Buckner, Nollau and Kastle, '15, Osborne and Mendel '16, McCollum, Simmonds and Pitz '17, Hogan '17, Lewis and Root '20, Sure '22, Champy and Gley '23.

Since d-lysine gave no evidence of influence on developmental growth in Obelia save the proliferation phase, the which will be shown as non-specific to lysine, and since growth resumption in rats after readmission of the compound to the diets deficient therein can not be attributed to proliferation enhancement save to negligible degree, it is valid to consider that mass increment or anabolism (Hammett, '36d) is the growth phase in which lysine is specifically significant. Sustaining this conclusion is the report of Outhouse and Krouse ('35) that rats fed lysine free diets may increase in stature but not in weight.

Now the only way in which an influence of size or weight increment could be derived from the Obelia data would be through exhibition of a general developmental enhancement. But such did not occur. While it is conceivable there might have been an increase in colony substance without increase in developmental expression one has the right to expect, on the other hand, that enhancement of mass increment in such a continuously growing organism as Obelia would result in a secondary better developmental growth all along the line. Inclination is towards the belief that mass increment was no better than it should have been in the d-lysine cultures.

It would follow that d-lysine is not a stimulus to weight increase.

The vertebrate work does not prove that with adequate lysine present, additional amounts act to enhance growth. It simply shows lysine must be present for growth to proceed. That the obelia grew and flourished without lysine in the cultures simply means there was already adequate lysine naturally present. As might have been expected. The addition of lysine to the cultures did not enhance development in general. This would indicate there was no stimulation of substance increase. The vertebrate data are not contradictory. For the addition of lysine to the lysine deficient diet of rat 1900 in the 1914 studies of Osborne and Mendel (Osborne and Mendel, '14) did not bring about weight growth resumption. Indeed the weight curve shows a drop. It is therefore clear that lysine is not a stimulus to the mass increment phase of growth or anabolism. Consistent but not decisive is the report that the compound does not increase bacterial growth (Gordon and M'Leod, '26).

It is conceivable that the amino acid may be indispensable to anabolism without being a stimulus therefor. It may be an essential and specific participant in some intermediary enabling process. If this be so only sufficient to unlock need be present for growth in mass to proceed. More would not necessarily produce acceleration in increment. Supporting this concept is the report that lysine deficiency results in poor use of protein (Outhouse and Krouse, '35).

The hypothesis therefore presents itself that the significance of lysine to growth is through metabolism.

Now the rodent work showed that for growth to proceed in the presence of lysine—tryptophane must be present. And the converse is true that for growth to proceed in the presence of tryptophane lysine must be present.

The Obelia work has established that tryptophane is a specific retardant of catabolic activity (Hammett and Elliott, '35). That its role in growth is as an indirect forwarder of anabolism through a braking influence on catabolic

disintegration. If to this fact be added the justified assumption that lysine is an enabling agent for anabolism rather than a stimulus thereto, the picture tends to clarify.

Thus: no lysine—no tryptophane: no growth—loss in weight: because no enabling of anabolism and no brake on catabolism.

Lysine—no tryptophane: no mass growth—weight loss: even though anabolism is enabled—because no brake on catabolism.

Tryptophane—no lysine: no growth—no weight loss: because no enabling of anabolism—but brake on catabolism.

Lysine—tryptophane present: mass growth: because enabling of anabolism and brake on catabolism.

Increase in tryptophane—lysine present: increase weight growth: because increase in break on catabolism—anabolism enabled.

Increase in lysine—tryptophane present: no acceleration mass increment: because no stimulation of anabolism—just simple enabling thereof plus brake on catabolism.

The allocation to lysine and tryptophane of metabolic function in relation to growth indispensability as derived from the Obelia data thus has the virtue of affording a basis for interpretation of the rat feeding experiments, the which has hitherto been lacking.

Consistent with the postulate that d-lysine is specific to mass growth is the report that the compound fed to lysine-deprived tadpoles gives proportionate size growth in distinction from the reaction to thyroid feeding where only metamorphosis results (Champy and Gley, '23). This suggests lysine has no specific role in developmental growth as such. The assumption is directly supported by the Obelia data and sustained by the report that the compound inhibits elastic fiber formation in tissue culture (Odiette, '32).

Further evidence that its growth function is allied to metabolic activity is the regression sensitivity of complete Obelia hydranths and the character of the regression curve produced by increasing concentration (Chart 1). These phenomena indicate that d-lysine is peculiarly effective in some phase of metabolic activity (Hammett, '37) and that the activity affected is different from that associated with expression of specific dynamic action (Hammett, '36 a, b, c). This allows assumption that d-lysine acts on a specific metabolic process. If as the other data suggests this is an enabling process for anabolism it is not impossible that excitation of such in Obelia would be expressed by increased regression.

This then puts d-lysine in the group of amino acids which are significant to growth because of their specific influence on some metabolis process, as distinct from those which act directly and specifically on some phase or other of development. It associates lysine with intermediary metabolic rather than direct utilization processes.

Turning now to the developmental growth responses to d-lysine, for such occurred, the data show proliferation was enhanced in its presence. Consistent with this is the report of mitotic flare-up when the amino acid is readmitted to the diet of tadpoles (Champy and Gley, '23), and the report that deep-

seated disturbance in sulfur utilization is productive of disturbance in lysine metabolism (Rosenfeld, '20).

In addition the data show that expression of differentiation is definitely held back. Consistent is the report that elastic fiber production—which may be taken as expression of differentiation—is also inhibited by the compound (Odiette, '32).

These reactions are not obviously specific to d-lysine. They have been observed to greater or lesser degree with d-arginine (Hammett and Chatalbash, '35), uracil (Hammett, '36e), thymine and cytosine (Hammett and Lavine). Since all of these compounds are definitive components of nucleo-protein it may be assumed for the present that the similarity is not to be taken as evidence that all act and act alike on the same proliferation and differentiation process: but that it is simply the result of an increased supply of nucleus-building materials.

Consistent with this is the report of Ugata (Ugata, '26) that Na-nucleate plays an important part in cell division, and the studies of Caspersson on the relation between cell division and nucleic acid (Caspersson, '24). Detailed exposition of the growth reaction to this group of compounds is reserved for a later paper.

Some attention has been given to the participation of lysine in neoplasia. While Courrier and Coste ('34), Voegtlin, et al ('36), and others report lysine deficient diets check such pathological growth, Resedale ('28) finds less than usual amounts in tumor tissue. All of which may or may not mean anything. In view of the demonstrated differentiation retarding effect of d-lysine it would be interesting to see if its withdrawal from tumors or diets of tumor bearing animals would yield a smaller growth of greater than control degree of differentiation, and thus a tumor of less potential malignancy.

SUMMARY AND CONCLUSION

One hundred eight experiments with more than fifty thousand Obelia hydranths, half of which were exposed to d-lysine in concentrations ranging from M/1,000,000 to M/6,250 show that this amino acid is not a stimulus to "growth-as-a-whole."

Although expression of the proliferation phase of developmental growth was enhanced and the differentiation phase held back, these reactions are not specific to lysine. They have been produced by other characterising components of nucleo-protein.

The metabolic activity productive of hydranth regression is increased by d-lysine in a quantitatively and qualitatively specific manner. This and correlated data suggest the specific significance of the compound to growth is through its influence on metabolism rather than through direct determination of any developmental growth activity.

The conclusion is advanced that d-lysine is essential to the mass increment or anabolic phase of growth, and this not as a direct stimulus thereto but as a participant in some intermediary enabling process therefor.

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THE INHIBITING EFFECT OF CORPUS LUTEUM ON THE GROWTH STIMULATING ACTION OF OESTRIN*

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In earlier experiments Loeb has distinguished between the follicular and the lutein phase of the sexual cycle, the former being under the domination of a growth substance given off by the growing and mature follicles, and the latter being controlled by a substance or substances given off by the corpus luteum (Loeb, '23a). But in addition to the independent action of these two substances during successive periods of the sexual cycle, it could be shown that they interfered with each other's action in certain respects. First, it was found that the corpus luteum prevents ovulation (Loeb, '11); this inhibiting action is effective during the ordinary sexual cycle as well as during pregnancy, and it could be demonstrated still more strikingly in the hysterectomized guinea pig, in which the corpus luteum persists at least as long as, or even longer than, during pregnancy. Extirpation of the corpora lutea during the early part of pregnancy may lead to a new ovulation notwithstanding the presence of a living embryo and placenta (Loeb, '14).

However, it could be shown that the action of corpus luteum not only inhibits ovulation, but also inhibits proestrous and the growth processes which substances given off by the mature follicle call forth, especially in the vagina (Loeb, '23a, b). During the ordinary sexual cycle, during pregnancy, and following hysterectomy in the guinea pig, growth and maturation of the follicles are not prevented, but the oestrogenic substances given off by the mature follicles are not able under these conditions to produce the typical growth processes in the vagina. On the other hand, extirpation of the corpora lutea during the sexual cycle leads to an acceleration of ovulation and of the preoestral proliferation in the vagina. On the basis of these data Loeb concluded that the corpus luteum inhibits not only ovulation, but also the growth effects which the follicular hormone, given off by the mature follicles, causes in the vagina.

Moreover, it could be observed that when, during early pregnancy, the corpora lutea are extirpated in the guinea pig and a new ovulation occurs without interruption of pregnancy, the normal predecidual changes in the uterine mucosa do not occur. We concluded therefore (1914) that during pregnancy there must be active an additional factor which prevents growth processes in the uterus.

These data suggested to Loeb and Kountz ('27), ('28) that if the corpus luteum inhibits the action of the follicular hormone (oestrin) which is given off

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under natural conditions, it should have a similar effect if this hormone were injected experimentally. Accordingly, they undertook an extensive series of experiments in which they injected follicular fluid or extracts containing oestrin into guinea pigs under various conditions; in some of these, corpora lutea were active, while in others they were lacking. They used for this purpose immature, normal, as well as underfed, and also castrated guinea pigs; in all of these animals corpora lutea were lacking. Furthermore, they injected guinea pigs during the ordinary sexual cycle in which the cyclic corpora lutea were active, and also pregnant and hysterectomized guinea pigs in which a long-persistent corpus luteum functioned. In these experiments they employed three different preparations of oestrin which possessed different potencies. Because the amounts of hormone which were at their disposal at one time were not large, they were not able to standardize the preparations by means of animal experiments. But inasmuch as these investigators were able in each case to compare the effects of oestrin in controls and in the experimental animals, it was possible to determine with certainty the effects of oestrin in guinea pigs in different states of sxual activity and thus it was possible to arrive at definite conclusions concerning the antagonism between the lutein and follicular hormones. The results obtained confirmed essentially the earlier conclusions of Loeb, since it was shown that the corpus luteum gives off a substance which inhibits the effect of oestrin on the growth of the vagina; this action was stronger in the case of the persistent than in the case of the cyclic corpus luteum, although it was present in the latter case also, especially during the period from about three to seven days following ovulation. However, Loeb and Kountz found that under conditions of marked underfeeding the growth reaction of the vagina to oestrin was diminished in intensity or missing altogether. These experiments were begun in 1924 and completed in 1926.

In 1927 Parkes and Bellerby showed that during the period of lactation in the mouse, oestrin does not elicit the vaginal reaction with the same readiness as in castrated animals; they attributed this effect to the presence of a corpus luteum during this period, and Simonnet ('27) likewise found that in the presence of an active corpus luteum injections of folliculin do not exert this characteristic effect on the vagina (Loeb, '23a, '23b). Subsequently, Courrier showed in the guinea pig that also the cyclic corpus luteum which develops postpartum in this species, inhibits the action on the vagina (Courrier, '30a), and Parkes ('29) observed that after elimination of the corpus luteum in the lactating mouse the inhibition of the oestrin action disappeared. F. W. M. Allen and R. K. Meyer found that injection of corpus luteum extracts from which oestrin had been removed inhibited the action of oestrin on the cornification of the vagina; the inhibiting action was the stronger, the greater the quantity of corpus luteum extract (Allen and Meyer, '35).

But, there is not only an inhibiting effect exerted by corpus luteum hormone of the action of oestrin; there is conversely also an inhibition exerted by oestrin on the action of corpus luteum. Loeb ('14) as well as Loeb and Kountz ('27) noted that injections of oestrin during the sexual cycle in guinea pigs prevented the predecidual reaction of the uterine mucosa which normally occurs between

the third and sixth day of the cycle. Similarly, Hisaw and Leonard ('30) found that when they injected oestrin and corpus luteum extracts simultaneously into castrated rabbits, oestrin prevented the occurrence of the predecidual epithelial reaction in the uterine mucosa. Correspondingly, Courrier ('30b) showed that in guinea pigs it is possible to prevent the formation of placentomata by injections of oestrin and, moreover, he succeeded in balancing the actions of oestrin and corpus luteum extracts in such a way that if the former substance predominated, the formation of placentomata, or in rabbits the epithelial predecidual reaction was inhibited, while the oestrin acted on the vagina in the usual way; but if the lutein substance predominated, the formation of squamous epithelium in the vagina was prevented, whereas placentomata formed normally.

In our new investigations which we carried out largely in 1933 it was our aim: (1) To determine the inhibiting action of the corpus luteum of pregnant and hysterectomized guinea pigs if standardized doses of oestrin have been given; (2) to determine at what period of the sexual cycle the inhibiting action of the cyclic corpus luteum is most effective; (3) to compare with the action of true corpora lutea the effect of pseudo-lutein formations such as develop in the guinea pig following injections of pregnancy urine and similar preparations (follutein), and (4) to obtain further data as to the inhibiting action of underfeeding on the proliferation of the vaginal epithelium. Therefore, we carried out six series of experiments in guinea pigs. Altogether 120 guinea pigs were used in these investigations. At the end of the experiment the sex organs of each animal were studied microscopically. The ovaries in each case were cut into serial sections and sections of vagina, cervix, uterus and mammary gland also were prepared from each guinea pig. Oestrin was prepared by us from human pregnancy urine according to the method of Doisy. Purification of the hormone was carried to the stage preceding crystallization. The substance thus obtained was dissolved in oil and injected subcutaneously. Rats were used for standardization. The results obtained in these experiments were as follows:

I. Series. Effects of oestrin in immature guinea pigs

Twenty-nine guinea pigs with initial weights varying between 175 and 205 grams were used. They received either one injection or three injections of oestrin (in oil) on three successive days. The doses of oestrin in different animals were 5, 8, 15, 25, 40, 60 rat units respectively. Examination took place either one or several days after the injections of oestrin had been completed.

A number of these guinea pigs approached or reached during the time of the experiment a weight which normally favored the development of large follicles, or even a beginning maturation. Notwithstanding this difficulty, it was possible to conclude that the injections of oestrin were effective and stimulated growth on the vagina the more so, the larger the dose of oestrin which had been given. In the vagina the proliferation of the cuboidal epithelium was slight with the smaller doses and became more marked with the larger doses of oestrin. In the uterus the effects were slight and in the mammary gland slight proliferation was usually noticeable with the larger doses. In guinea pigs in which there was

a loss in weight or a lack of a decided gain in weight during the course of the experiment, the vagina failed to show proliferative changes; this was especially noticeable when the ovaries had become hypotypical in consequence of inadequate diet.

II. Series. Effects of oestrin in guinea pigs during different phases of the sexual cycle

Guinea pigs were injected either on the second, fifth or ninth day of the sexual cycle with one dose of 10, 20, 30 or 50 rat units of oestrin. Examination took place three days after the injection. The initial weights of these animals varied between 320 and 385 grams.

Subseries a.—Eight guinea pigs were injected on the second day of the cycle and examination took place on the fifth day. In the ovaries good corpora lutea and large preserved, but not mature, follicles were present, while granulosa-degenerated follicles were absent, in accordance with the usual findings at this stage of the sexual cycle. Vagina and uterus likewise showed the characteristics of this stage of the cycle, the uterine mucosa being predecidual. Oestrin was without effect. The mammary gland, however, was proliferating in the injected guinea pigs.

Subseries b.—Eight guinea pigs were injected on the fifth day of the cycle and examined on the eighth day. In the ovaries besides corpora lutea, follicles in various stages of maturation were present. The vaginal epithelium showed slight proliferation, with mitoses in the cuboidal epithelium; only in one case two to three layers of cuboidal epithelium had developed. In the uterus the predecidual tissue was retrogressing. In the mammary gland proliferation occurred in the animals which had received the larger doses of oestrin. At this period there was, therefore, a slight stimulation of the epithelium of the vagina and some stimulation of the mammary gland.

Subseries c.—Seven guina pigs received one injection of 20, 30 or 50 units of oestrin on the ninth day of the sexual cycle and examination took place on the twelfth day. Maturing or mature follicles were seen in the ovaries. The corpora lutea showed some vacuolization in the peripheral cells. In the vagina slight stimulation similar to that observed in Subseries b was observed, but in some cases, especially in the animals which received the largest dose, 50 rat units, the proliferation progressed further, to the formation of typical squamous epithelium. There was, here, a proportionality between the intensity of the proliferation and the amount of oestrin injected. In the uterus no definite stimulation was noticeable, but in the mammary gland marked proliferation was found, which was probably due to oestrin given off by maturing or mature follicles. We find therefore that at later stages of the sexual cycle the effects of oestrin on the vagina were more marked than in earlier stages.

III. Series. Effects of oestrin in ovariectomized guinea pigs

Eighteen oophorectomized guinea pigs were used in this series. Twelve animals received one injection of 5, 10, 20, 25, 50 or 75 rat units of oestrin; four animals received three injections of oestrin on three consecutive days.

Two oophorectomized guinea pigs which did not receive oestrin served as controls. Examination took place one or three days following the injections, which were begun eleven to thirteen days following oophorectomy; but one animal was injected as late as two months subsequent to this operation. The initial weights of the majority of these guinea pigs varied between 400 and 600 grams.

In this series the effects of oestrin on the proliferation of the vagina were at least as marked as in the immature guinea pigs of Series I, although the weights were considerably greater in the oophorectomized animals. The effects were more marked in this series than in the guinea pigs injected during the earlier stages of the sexual cycle, and in the uterus and mammary gland they were more definite in the animals receiving the larger doses of oestrin.

IV. Series. Effects of oestrin in hysterectomized guinea pigs

In this series fifteen hysterectomized guinea pigs, in which however, very small parts of the uterus had been left behind, were used; two of these animals serving as controls were not injected with oestrin, while thirteen received either one injection or three injections of oestrin, one on each of three successive days. Those that received three injections of oestrin were examined one day following the last injection, while the others were examined three days following the injection. 10, 25, 50 or 75 rat units were the doses of oestrin used. In the large majority of the experiments hysterectomy had been carried out forty-four to fifty-one days previous to the administration of oestrin. In two injected guinea pigs and in one control examination occurred about three and a half months following hypsterectomy. The initial weights of these animals at the time of hysterectomy varied between 540 and 760 grams. There was usually during the experiment some loss in weight in these guinea pigs, which was however, slight in the majority of cases.

In the ovaries large, well preserved corpora lutea with thick-walled arteries were seen. In some of the corpora lutea slight peripheral, or in certain cases also more generalized vaculolization was noted, an indication that in some of these organs degenerative processes had set in, although other corpora lutea in the same animals were well preserved. Also remnants of completely degenerated corpora lutea were seen in some instances. There were usually large mature follicles present; they subsequently degenerated. In addition various other types of well preserved, as well as atretic follicles were found. In one of the guinea pigs, which had been hysterectomized three and a half months previously, there were indications that a new ovulation had taken place a short time preceding examination. In another guinea pig with an initial weight of 540 and an end-weight of 530 grams, which had received one injection of oestrin three days previously, in addition to well preserved corpora lutea, with thick vessels, mature follicles were seen, which had been converted into pseudocorpora lutea through growth of connective tissue and capillaries into the granulosa without a preceding ovulation having taken place. All other follicles, except the very small ones, had undergone atresia. The character of the young pseudocorpora lutea with their retained eggs and the lack of a typical reaction in the vagina,

as well as the presence of large, on the whole, well preserved corpora lutea, indicated that in this case no real ovulation but a somewhat related condition had occurred.

The vagina was in a resting condition in the greater number of these guinea pigs. The mucous membrane was usually covered by a layer of mucoid, high cylindrical epithelium, showing papillary arrangement; underneath this layer was one layer of flat cuboidal epithelium, in which in no case were mitoses seen. But in two animals, which had received three injections of oestrin, and in two animals, which had received, respectively, one injection of 50 and 75 rat units, two layers (and in one case probably three layers) of cuboidal epithelium were noted. We may therefore conclude that the preserved corpora lutea strongly inhibited proliferation of the vaginal epithelium which otherwise would have taken place, but that they were not able to prevent it entirely if the oestrin action had been strong. The mammary glands were large and consisted of many acini which were mostly small. Mitoses were seen, as a rule, but their number differed in different guina pigs and also in different areas of the same animal. The greatest mitotic action in the acini was observed in two guinea pigs which had received the largest doses of oestrin. In several cases living placentomatous tissue was found at the cut ends of the uterus about seven weeks following the almost complete hysterectomy; in these instances the incisions into the uterus had been made during the luteal phase of the cycle. Sometimes large areas of placentomatous tissue, including the characteristic pearls, were preserved and the living tissue was, in places, arranged in the form of a shell around the central necrotic material. The cells situated around the blood vessels were least differentiated, while the preserved areas adjoining the necrotic zones often contained giant cells with giant nuclei. Lack of favorable conditions of nourishment, including lack of oxygen, in cells which were under the influence of growth stimuli, led to abnormal growth manifesting itself in differentiation and giant cell formation; but in areas where conditions were more favorable, such as were found in the neighborhood of blood vessels, mitotic division of the cells still continued, to take place, although only a to a slight extent. However, in general, the greater parts of the placentomatous tissue had already become necrotic by this time. No placentomata were seen in the guinea pigs examined as late as three and a half months after hysterectomy.

We may then conclude that the presence of old corpora lutea, whose retrogression had been prevented through hysterectomy, inhibited the full effect of oestrin on the vagina.

V. Series. Effects of oestrin in guinea pigs injected with human normal or pregnancy urine

Subseries a.—Four guinea pigs with an initial weight varying between 180 and 190 grams received daily intraperitoneal injections of 3 cc. normal human urine on five successive days; on the sixth day 10, 20, 30 or 50 rat units of oestrin were injected subcutaneously. Examination took place three days following the

administration of oestrin. In all of these guinea pigs large, preserved follicles were visible, but only in the animal which had received 10 rat units of oestrin were mature follicles seen. The vagina showed in every case some proliferation, which was only slight in the guinea pig which received 10 units of oestrin, but which increased with increasing doses of this hormone. 50 units caused development of well formed squamous epithelium, but without keratin. Mitoses in the cuboidal layer were found in all animals, except in the one receiving only 10 units of oestrin. The connective tissue of the uterine mucosa showed signs of stimulation in the guinea pigs which received 20, 30 and 50 units. The largest dose of oestrin caused, perhaps, also a small increase in the height of the uterine epithelium. In the mammary glands there was either slight proliferation or signs of secretion were visible.

We may then conclude that injection of normal urine does not inhibit the action of oestrin on vagina and uterus.

Subseries b.—Eleven guinea pigs were injected intraperitoneally with five doses of 3 cc. human pregnancy urine, one dose being given daily. This period was followed by a series of three injections of oestrin (5, 8, 15, 25, 40 or 60 units) on three successive days. Concomitantly with the last two injections of oestrin and on the day following the last oestrin injection, again three doses of 3 cc. pregnancy urine were administered. The animals were examined between the first and fourth day following the last injection of oestrin. The initial weight of these guinea pigs varied between 175 and 180 grams, and the last weight varied between 180 and 235 grams.

In the ovaries, interstitial gland bodies and follicles in the stage of luteinizing connective tissue atresia, as well as transitions between these structures and pseudocorpora lutea, and, in addition, interstitial gland cells developing in the medulla were seen. The ovaries of the guinea pig receiving 25 units of oestrin consisted mainly of large interstitial gland bodies which were separated from each other by thin fibrous bands. The size of the follicles in general corresponded to the changes in weight which the animals underwent during the experiment. In a number of these guinea pigs only medium sized follicles developed, while in others some follicles reached a large size; but mature follicles were not observed.

The vagina was covered by a cylindrical mucoid surface epithelium. The cuboidal epithelium underneath this layer was either in a resting condition, or showed signs of a very slight proliferation, as was indicated by the presence of a very small number of mitoses; even with the largest dose of oestrin no marked proliferation was obtained. In the uterus the surface epithelium was usually high, and in certain animals some mitoses were seen in the connective tissue. The mammary gland varied: in some animals there were signs of a slight proliferation; in others the gland was in a resting state.

We may then conclude that the presence of luteinized structures in the ovaries, other than typical corpora lutea, prevented or very much restricted the proliferation of the vaginal epithelium, which otherwise would have occurred under the influence of oestrin injections. On the other hand, there were signs

of stimulation of the uterine mucosa and of the mammary gland in some cases. The pseudolutein structures, which develop in the ovaries under the influence of injections of pregnancy urine, inhibited, therefore, the action of oestrin on the vagina in a similar manner to the true corpora lutea, which are characteristic of hysterectomized guinea pigs.

Subseries c.—In this series of eight guinea pigs with initial weights of 175 and 180 grams, the mode of experimentation was similar to that used in Subseries b, except that only one dose of oestrin was given following a series of five injections of 3 cc. human pregnancy urine. In the majority of cases the injection of oestrin was followed by three additional injections of 3 cc. pregnancy urine, one injection being given daily.

In the ovaries, interstitial gland bodies, showing in some cases transitions to pseudocorpora lutea, follicles with luteinizing connective tissue atresia and occasionally smaller follicles with premature maturation of the granulosa were observed. No proliferation of the vaginal cuboidal epithelium was obtained; the upper layer of epithelium consisted of a papillary layer of high cylindrical vacuolar cells. No definite changes were noted in the uterus, but in the mammary gland there was usually a moderate proliferation of cells.

These results confirm those obtained in Subseries a; both indicate that pseudolutein formations inhibit the growth-promoting effect of oestrin on the vagina about as effectively as do true corpora lutea.

VI. Series. Effects of oestrin in guinea pigs injected with follutein*

Twelve guinea pigs received each five doses of 0.2 cc. follutein on five successive days; on the day following the last injection one dose of oestrin (8, 10, 15, 25, 40 or 60 rat units) was given to seven guinea pigs. Examination took place three days after the last oestrin injection. Instead of one, three doses of oestrin were given to five guinea pigs; these animals were examined from two to four days after the last oestrin injection. Two additional guinea pigs, serving as controls, received only five injections of follutein. The original weights of these animals varied between 175 and 190 grams; during the course of the experiments some of them gained weight, the greatest weight reached being 220 grams, while some lost slightly in weight.

In the ovaries of these animals interstitial gland bodies, some of which showed transitions to pseudocorpora lutea, follicles in the process of luteinizing connective tissue atresia, as well as smaller or larger pseudocorpora lutea, were found. Not all these structures occurred in every animal. Moreover, the amount of interstitial gland tissue varied in different guinea pigs. In the five guinea pigs injected with three doses of oestrin each, only small or even rudimentary interstitial gland bodies were seen. The largest size of follicles attained varied in different cases and there was, in general, a relation noticeable between the changes in weight observed in the animals during the course of the experiments

* We are indebted for the collection used in these experiments to Dr. J. F. Andersen, Director of the Biological Laboratories of E. P. Squibb and Sons.

and the size of the follicles reached. Vagina, uterus and mammary gland of the control guinea pigs were in a resting state.

In the animals injected with one dose of oestrin the vagina showed slight proliferation in one guinea pig, while squamous epithelium without keratin was found in another guinea pig. In two of the guinea pigs which received three doses of oestrin, squamous epithelium covered by mucoid cylindrical epithelium formed. In the animal which received the largest dose of oestrin the lack of proliferation in the cuboidal epithelium of the vagina was probably due to the loss of weight which occurred in this guinea pig during the course of the experiment. Uterus and mammary gland in some of the animals in this series showed no stimulation, while signs of slight stimulation were present especially in those guinea pigs which received the largest amounts of oestrin.

In this series the inhibition of proliferation in the vagina, while noticeable, was less marked than in the animals which received pregnancy urine, or which had been hysterectomized; this was, perhaps, due to the fact that the luteinizing effect of follutein on the ovaries of the guinea pigs was, on the whole, not quite as pronounced as that obtained in the IV, and V Series, in which lutein structures had been produced by other means.

SUMMARY AND CONCLUSIONS

These experiments confirm our previous finding that during the sexual cycle at a time when the cyclic corpus luteum is active, the growth-reaction of the epithelium of the vagina which is called forth by oestrin in the immature and castrated guinea pig, is inhibited. This inhibition seems to be most marked about the middle of the cycle, at a time when the predecidual uterine growth-reaction has about reached its height. In accordance with our previous observations, the persistent corpus luteum of the hysterectomized guinea pig exerts a very strongly inhibiting effect on the oestrogenic growth of the vagina. It resembles in this, as in other respects, the corpus luteum of pregnancy (Loeb, '17, '23, '27). In regard to the latter, Courrier ('29) suggested that inasmuch as it can be extirpated during the latter part of pregnancy without an interruption of the pregnancy occurring, this organ is not active at that time, and that the inhibition of the oestrogenic growth effect cannot therefore be attributed to it. While it is very probable that during this period a constituent of the placenta assumes the function of the corpus luteum, this does not exclude the possibility that also the latter organ continues to function and that there exists a double mechanism safeguarding the continuation of the pregnancy at this time. This conclusion is the more likely, because our experiments have shown that in the hysterectomized guinea pig the corpus luteum exerts its growth-inhibiting function at least during as long a period as corresponds to the duration of pregnancy. Various pseudolutein structures which develop in the ovary of the guinea pig, under the influence of human pregnancy urine, exert a similar inhibiting effect on the oestrogenic growth of the vagina to that exerted by the true corpus luteum. This is in accordance with observations which we have

made previously concerning the incompleteness or lack of proliferation in the vagina under various conditions in which these pseudolutein formations were found in the ovary. While in the majority of these structures both granulosa and theca interna derivatives are present, the proportion of the latter is, on the average, relatively much greater in the pseudolutein formations than in the true corpus luteum where the transformed granulosa cells largely preponderate.

The inhibiting action of lutein hormone on the oestrogenic growth of the vaginal epithelium does not consist in a direct mutual neutralization of the two kinds of hormones involved, inasmuch as the growth of the mammary gland is not inhibited under these conditions. It depends probably on a state refractory to the action of oestrogenic hormone, which is induced in the vaginal epithelium by a substance given off by the lutein structures.

While the inhibition exerted by such a substance on the oestrogenic growth of the vagina is a specific one, a similar inhibition of the oestrin effect which is caused by the undernourishment of the guinea pig is of a non-specific, character. The mechanism underlying the latter type of inhibition may be of a twofold nature: (1) Growth of vagina as well as of other tissues requires an adequate food supply. (2) Undernourishment may lead to changes in the structure and metabolism of the vaginal epithelium, which makes it less responsive to growth stimuli.

In the hysterectomized guinea pigs another growth phenomenon of some interest could be observed. In a number of the animals considerable parts of the placentomata, which developed as the result of the incisions made into the remnants of the uterine mucosa during the lutein phase of the cycle, remained alive as late as seven weeks following the operation; this applies especially to placentomatous tissue which was situated around the blood vessels. Around the blood vessels these cells remained relatively undifferentiated, whereas in the neighborhood of necrotic tissue abnormal differentiation took place in the placentoma cells under the influence of growth stimuli acting on this tissue after it has been placed in an unfavorable environment.

There have been observed in recent years a number of specific growth inhibiting substances. As an example we may cite the antagonism which exists between the growth of the thyroid stimulating hormone of the anterior pituitary gland on the one hand, and on the other hand, iodine and thyroid hormone which inhibit the action of the anterior pituitary hormone (Loeb, '32). The antagonism between lutein and oestrogenic hormones is a further example of a mutual growth inhibiting action exerted by two substances, each one of which, when acting alone, functions as a growth stimulator.

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SULPHYDRYL AND RADON INDUCED NECROSIS*

By

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That there is positive correlation between the sulphydryl group (−SH) and cell increase in number was first established by Hammett ('29a, '31). Sulphydryl compounds such as thiocresol, benzyl mercaptan, and other allied substances have been employed in studies on growth, particularly in the healing of ulcers, bed-sores and the like (Hammett and Reimann '29, Reimann and Hammett '29, Reimann '29, Brunstig and Simonsen '33). Hammett ('30) has postulated and demonstrated by many experiments (Hammett and Hammett, '32a, b, and Hammett '33a, b) that the $(-SH):(-SO_3)$ equilibrium regulates cell proliferation and that the (−SH) group is a naturally occurring stimulus to cell increase in number, while its partially oxidized derivatives are the naturally occurring retardants thereof. The work of Lavine ('35) substantiates this.

Proliferation alone, according to Reimann and Hammett ('35) and Hammett ('35) is not productive of malignancy. In the light of Warburg's ('30) theories and findings regarding the metabolism of normal and cancer cells it may be that the characteristic glycolysis and changes in oxidation of the cancer cells might be reflected to the sulphydryl group. A most comprehensive survey of this matter is to be found in two papers by Reimann ('31, '32). Until further work is done, however, it is questionable whether growth stimulating substances should be applied to malignant lesions or to premalignant lesions. It is probable that such applications might stimulate malignant tissue proliferation as suggested by Hammett ('29) and others (Voegtlin et al, '36). Theoretically then the application of sulphydryl preparations to wounds on patients of the cancer ages might be contraindicated, though Reimann and Hammett ('29), Riemann('30) and Brunstig and Simonsen ('33) have used such sulphydryl containing substances in the treatment of ulcers with good results.

That a study of growth stimulating substances may lead to the discovery of materials having growth inhibitory powers is evident from the report of Hammett ('30) and his co-workers ('33, and Lankenau '36). It is apparent from our findings that the partially oxidized form of the (−SH) group as found in (S:S) as cystine is essentially inactive in promoting growth. This is consistent with a like report made by Hammett ('29a) from his work with root-tips. It would also seem that materials capable of promoting the oxidation of the (−SH) group would be indicated as probable growth inhibitors. One such—radium—has been shown to be so active Hammett ('32). Various other authors have shown that sulphydryl compounds are more or less sensitive to radiating energy, Hueper et al ('33). The oxidation-reduction mechanism of tissues may be primarily influenced by x-rays and radium rays. Other ways in which the oxidation-reduction

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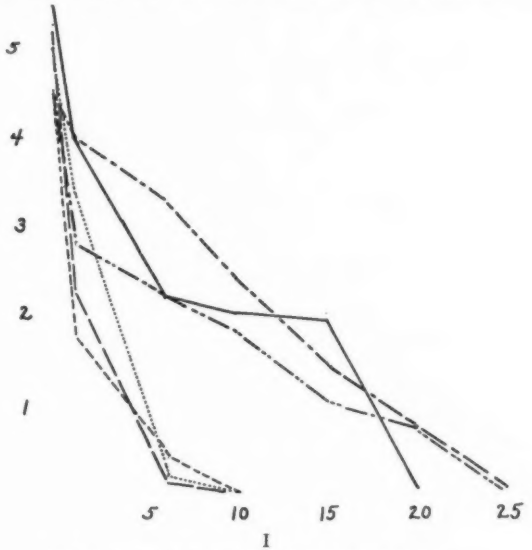
mechanism of cells might be influenced by irradiation are through the effects on cell ferments or interference with the dispersion of colloidal proteins.

EXPERIMENTAL

It became apparent early in our work that the healing rate of wounds, uncomplicated by infection, was not appreciably affected by the application of growth stimulating material. We took this to mean that the healing rate or



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Abscissa, wound size in millimeters, long diameter.
ordinates, time in days.
Cysteine treated wounds, Iradiated ————————
Not irradiated — — — — — —
Cystin treated wounds, Irradiated—— – —— – —— – ——
Not irradiated. . . . . .
Saline control wounds, Irradiated—— . . —— . . —— . . ——
Not irradiated —— —— —— ——

response in normal experimental animals bearing standardized untreated wounds was maximal. It thus seemed essential that the standard size wounds in the experimental animals used (guinea pigs) should have the normal healing process delayed in some manner. We believe we have obtained such a healing delay by the implantation of gold seeds containing radon. By this procedure an ulcerating wound resulted during the decay period of the radon within the gold seeds.

The preparation and treatment of the guinea pigs was carried out as follows:—the animals were lightly etherized. Four millimeter incisions were made in each femoral triangle. The right femoral area was implanted with four dead radon seeds. The left femoral area was implanted with four radon seeds each of which

contained from .7 to 1. millicuries. To prevent the loss of seeds they were placed beneath the fascia and along the femoral vessels. Twelve animals were prepared in this manner. An additional four animals had four dead radon seeds in the right and four dead seeds in the left femoral triangle regions. A single silk suture was used to approximate the edges of the wounds. All applications were made at least once daily as it was found quite impossible to keep wet dressings on the animals. Four of the animals whose left femoral triangles contained an average of 3.09 millicuries of radon received normal saline applications. Four others of the irradiated group received applications of a one per cent suspension of cystine to the wounds. The healing rates in these two groups were similar. In a third group of four radon treated animals the irradiated and non-irradiated wounds were treated with one per cent solution of cysteine hydrochloride, the acid group of the solution being neutralised with sodium hydroxide just previous to the applications. The hydrogen ion concentration of the solutions was near 7. About 6.3 cc. of normal sodium hydroxide solution were added for each gram of cysteine hydrochloride. The right femoral triangle containing the dead seeds showed no appreciable acceleration of healing. The left triangle wounds healed on an average of twenty days from the wound preparation. This was about five days earlier than the saline or cystine treated irradiated wounds of the other groups. An additional group of four animals had incisions prepared without implants being made. The left side wounds treated with freshly neutralised cysteine hydrochloride solutions and the right side wounds treated with normal saline solution, no healing acceleration was noted on either side.

The results are displayed in the chart, each curve represents the results from four animals as averaged.

DISCUSSION

It is possible that organic sulphur compounds do play a role in the development and growth of epidermal tissue since keratin and hair normally contain more cystine than other tissues. The epithelial tissue is supposedly more capable of repairing injuries than is connective tissue. These considerations fit in with the assumption that carcinomas are more numerous than are sarcomas and the epithelial tissues are more apt to be injured than connective tissue. Sulphur to be concerned in tissue growth must be in a reactive form as sulphydryl (−SH). Klauder in the discussion of Brunstig and Simonsen's ('33) findings, believes that the form of sulphur in the nucleus during mitosis is of importance. At any rate the cysteine sulphur is one of the most reactive points of the protein molecule. Mathews (23) has postulated that the fibers of the aster in cell division might be formed through protein sulphur linkages. He also cites the early attempts of Heffter and of Hopkins, as well as his own work, in trying to bring the sulphur linkages into relationship with cell processes. Purified growth promoting extracts from embryonic tissues have proven to be non-antigenic, Owen and Cutler (unpublished). The growth stimulating elements of these extracts are thus more simple than a complete protein.

From the chart it appears as if the cysteine treated and irradiated wounds

were on the average retarded more in healing rate, than similar wounds to which either saline or cystin were applied, during the period of the radon activity. This may indicate that actual growth retarding substances were formed from the cysteine (−SH) but are not formed from (S:S) cystin by radon on tissues. Attention is also invited to the fact that when the radon activity is fading that great growth stimulation occurs by the application of cysteine (−SH). The interpretation here is obviously this, radon changes or destroys (−SH) and renewal by application of cysteine after radiation evinces definite growth stimulation.

Other materials have been reported as important in the stimulation of healing rate, viz. Greenbaum ('36) allantoin; Stewart ('34) calcium salts; Lafresniere ('36) dextrose. These are physiological and available from tissues. Extracts of many types of tissues, endocrine preparations and complex chemical mixtures have been tried for growth stimulating effects. Very few of these have been tried on tissue cultures on which metabolism determinations and chemical changes were noted so as to indicate the mechanism of growth stimulation. It is probable that the application of physico-chemical laws as done by Beutner ('33) will lead to a greater understanding of the phenomena of growth.

SUMMARY

1. Sulphydryl compounds as cysteine stimulate normal tissue repair following radon induced necrosis.

2. Cystine or saline solution was shown to be ineffective in stimulating healing rates of necrosed wounds or of normal wounds.

3. Radon apparently changes sulphydryl groupings to depress the growth stimulation and possibly forms growth retardants.

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DEVELOPMENTAL GROWTH AND NUCLEIC ACID COMPONENTS—IV. THYMINE

By

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This is a report of an experimental evaluation of Thymine in terms of the developmental growth of Obelia geniculata. *There is evidence presented which indicates this component of nucleic acid is significantly concerned in some process essential for expression of proliferation and differentiation.*

This report is extension to thymine of the general inquiry into the part played in growth by the naturally occurring tissue components of general distribution.

Obelia geniculata was the test animal. Its description together with that of the experimental procedure are given in reports referred to earlier (Hammett and Schlumberger '37).

The thymine as prepared by the junior author from sheep thymus had the chemical and physical properties essential for identity and purity. Stock solutions containing 0.056 gram per 89 cc. fresh sea-water provided daily the aliquots from which test culture solutions of desired concentrations were made.

Sixty-eight experiments were run with close to 35,000 animals, half of which were exposed to the compound in concentrations ranging from M/400,000 to M/6250. Tests and controls were run simultaneously under like conditions of temperature, illumination and pH. Four changes into fresh sea-water were made during the 24 hour course of experiment.

The distribution of the material and the results of the trials are given in Table 1. The method of its derivation, construction, and analysis have been given elsewhere (Hammett and Schlumberger).

RESULTS

The table gives no evidence that thymine was toxic to Obelia in any of the stated concentrations. Nor is definite evidence found of influence on maintenance or regression. Hint of lessened catabolism is had. Test expression of the activity was less than control in 35 and greater in but 27 of the 68 trials. By concentration it was less in 4 and greater in but 2 of the 7 series.

The outstanding growth reaction was a definite enhancement in expression of proliferation and differentiation activity. Thus—

Bud change to half in the tests was greater than control in 24 and less in but 15 of the 56 expressions given by the seven sets of experiments; by concentration it tended to be greater in four and less in but one of the seven series: and when the data are combined—test is greater than control in four and less in but two of the eight proliferation potentialities.

Test differentiation was greater than control in 26 and less in but 15 of the

TABLE 1

TREND OF TEST DEVIATION FROM CONTROL WITH RESPECT TO DEVELOPMENTAL AND METABOLIC ACTIVITY IN THYMINE CULTURES. ALSO THE NUMBER OF EXPERIMENTS, COLONIES, HYDRANTHS, AND GONOPHORES AT THE STATED CONCENTRATIONS

M/1000×	400		200		100		50		25		12.5		6.25	
No. Experiments	10		10		10		10		8		10		10	
	Cont	Test	Cont	Test	Cont	Test	Cont	Test	Cont	Test	Cont	Test	Cont	Test
No. Colonies	150	150	150	150	142	142	150	150	120	120	150	150	150	150
No. Hydranths	2602	2604	2573	2572	2507	2513	2645	2641	2004	2001	2557	2551	2591	2592
No. Gonophores	106	150	196	194	106	137	171	149	65	69	80	68	108	83
						Growth								
	+ − =		+ − =		+ − =		+ − =		+ − =		+ − =		+ − =	
Initiation	2 2 3		1 3 3		2 1 4		2 1 4		2 4 1		2 3 2		3 1 3	
Proliferation	3 1 4		4 2 2		6 2 0		2 4 2		1 2 5		6 1 1		2 3 3	
Differentiation	5 4 1		5 0 4		3 3 3		4 2 3		2 3 5		3 1 6		4 2 4	
Organization	5 1 3		0 4 4		3 1 4		2 3 3		2 3 4		3 0 6		1 3 4	
						Metabolism by Single Expression								
Maintenance	5 3 2		5 5 0		1 8 1		3 5 2		3 4 1		3 6 1		5 4 1	
Catabolism	3 4 3		7 2 1		4 6 0		1 8 1		6 2 0		4 5 1		2 8 0	
						Metabolism by Combined Expression								
	Cont	Test	Cont	Test	Cont	Test	Cont	Test	Cont	Test	Cont	Test	Cont	Test
Regression	34.7	34.1	29.1	29.6	19.4	22.0	34.2	33.9	35.3	34.3	35.8	37.6	44.6	43.9
Catabolism	85.3	86.5	83.0	84.6	67.1	67.1	77.3	72.6	84.2	86.1	84.0	83.3	82.2	76.9
						Increase in Percentage Regression								
	−1.7		1.7		13.4		−0.9		−2.8		5.0		1.6	

70 expressions given by the seven sets of experiments; by concentration it tended to be greater in four and less in but one of the seven series; and when the data are combined—test is greater in seven and less in but one of the ten potentialities for differentiation activity expression.

Initiation, on the other hand, and organization too, gave no open response. Thus:

Bud formation in the tests was greater than control in 14 and less in 15 of the 49 expressions given by the seven sets of experiments; by concentrations it tended to be greater in three and less in two; and when the data are combined—test is greater in three and less in three of the seven initiation potentialities.

Organization in the tests was greater than control in 16 and less in 15 of the 63 expressions given by the seven sets of experiments; by concentrations it tended to be greater in three and less in two; and when the data are combined—test is greater than control in two and less in three of the nine potentialities.

DISCUSSION

The data show the developmental activities of proliferation and differentiation to be specifically enhanced in the presence of thymine.

It would be nice to write that the increased expression of differentiation was a consequence of increase in the antecedent activity of proliferation. That such sequence may occur has been demonstrated by the rodent skin reaction to the proliferation stimulating influence of sulfhydryl (Hammett, '31). The converse is conceivable. Unfortunately, however, no correlation can be traced between occurrence of increase in proliferation and occurrence of increase in differentiation in the 56 pairs of observations provided by the data.

This leads to the idea that thymine may be of separate significance to each of the two activities.

From the fact that proliferation tends to be greater in the presence of other components of nucleo-protein such as lysine (Hammett, '37), arginine (Hammett and Chatalbash '35), uracil (Hammett, '36), and cytosine (Hammett and Lavine '37), and the fact that nucleic acid is a forwarder of cell division (Ugata '26, Casperson '36), it may be assumed the like response to thymine is expression of group rather than specific influence. Possibly it is simply reaction to plethora; possibly it comes from a common by-product of intermediary metabolism; possibly it indicates a constellation of separate processes each factored separately by and individual component of the group (Hammett '36b). Further exposition is reserved for later analysis.

The differentiation increase could well mean that thymine as such is a specific participant in some process essential to this developmental activity. On the other hand since the two dicarboxylic acids, d-glutamic (Hammett 36c) and l-aspartic (Hammett and Schlumberger '37) have been found specific stimulants of differentiation expression, and since it is conceivable that dicarboxylic acids may result from the intermediary metabolism of these pyrimidine derivatives (Cerecedo '32), it may be that the reaction to thymine is not expression

of thymine specificity at all; but of dicarboxylic or derivative specificity. Demonstration for decision should not be difficult.

Regardless of eventual definition these data not only show thymine to have a definite influence on particular activities, but also they show that the picture drawn by the response of the several components of the growth constellation to its presence is peculiar to the compound and like that of no other so far seen.

Thus—though thymine like its congenor uracil produces no marked regression or evidence of specific dynamic effect; though like uracil it produces a possible very slight trend to lessened catabolism, and an increased expression of proliferation: it differs distinctly therefrom in that it does *not* act as a stimulus of recurrent growth, and it does act to enhance differentiation expression rather than to retard as was the case with uracil.

It has been recorded that the presence of an added CH_2 group is accompanied by a tendency to lesser regression increase (Hammett and Schlumberger). It may or may not mean anything, but it is worth recording that whereas test regression did not tend to be less than control in any of the six sets of uracil experiments but did tend to be greater in five; in the case of thymine with the H of position five replaced by CH_3 there occurred a tendency to a lesser test regression in four of the seven sets and a greater in but three. These derive from the percentage regression values of the combined data of each concentration set. They also appear in the reaction trend by single experiment. And they appear when all are grouped. Thus—test regression was greater than control in 54 and less in 30 per cent of the trials with uracil; while in the case of thymine it was greater than control in but 51 per cent and less in 37. Though the differences are small their deviation direction is consistent.

SUMMARY AND CONCLUSION

From 68 experiments with close to 35,000 animals half of which were exposed to thymine in concentrations ranging from M/400,000 to M/6250 it appears that the compound definitely accelerates proliferation and differentiation expression in *Obelia geniculata*.

Neither initiation nor organization are affected.

The compound has no marked influence on the changes associated with maintenance, regression, or specific dynamic action. It may induce a tendency towards slowing of catabolism.

The conclusion is that thymine is of particular significance to processes concerned in proliferation and differentiation.

The possibilities for and against this being specific are noted.

The work reported here was done at The Marine Experimental Station of The Institute, North Truro, Massachusetts, with the aid of Miss A. Bein and the technical assistance of Misses Chatalbash, Elliott, and Lavine, and Mr. Hans Schlumberger to whom our gratitude is hereby expressed.

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THE CONSEQUENCES OF SIGMOID GROWTH FOR RELATIVE GROWTH FUNCTIONS

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INTRODUCTION

It has been shown (see Huxley, '32) that the relative growth of two parts of an organism or of a part and the whole, conforms in a large number of cases to the parabolic function

$$y = bx^k. \tag{1}$$

This relationship is derivable, as Huxley ('32, p. 7) and others have observed, on the assumption that the parts x and y increase exponentially with time. That is, if we write

$$\begin{cases} y = a_1 e^{r_1 t} \\ x = a_2 e^{r_2 t}, \end{cases}$$

the elimination of t between these two equations yields equation (1) with $k = r_1/r_2$ and $b = a_1/a_2^k$. Schmalhausen's growth-quotient q is also derived in this fashion, and is thus identical with k in Huxley's equation (see Schmalhausen '30; also Huxley, '32, p. 141).

Equation (1) follows also if x and y are themselves parabolic functions of time, of the form

$$\begin{cases} y = c_1 t^{r_1} \\ x = c_2 t^{r_2}. \end{cases}$$

Here $k = r_1/r_2$ and $b = c_1/c_2^k$. In fact, a parabolic relationship between x and y results in any case in which

$$\begin{cases} \dfrac{dy}{ydt} = r_1 f(t) \\ \dfrac{dx}{xdt} = r_2 f(t). \end{cases}$$

Huxley ('32, p. 6) argues for the general biological validity of these differential equations; however, as Bernstein ('34) has pointed out, they are actually valid only as a special case. The most general form of such equations is given rather by expressions of the type

$$\begin{cases} \dfrac{dy}{ydt} = f_1(y, t) \\ \dfrac{dx}{xdt} = f_2(x, t) \end{cases}$$

where f_1 and f_2 may or may not be functions of the same general kind. It is obvious that these do not necessarily lead to (1).

Growth curves which extend over a relatively large portion of the growth period are most frequently sigmoid in character, rather than exponential or parabolic. The mathematical analysis of sigmoid growth curves is as yet in an unsatisfactory state; of the various equations which have commonly been applied, none has thus far been shown to have an adequate theoretical foundation. Nevertheless, it is of interest to determine the character of the relative growth functions derived on the assumption that the growth of the parts x and y is expressed by certain of these equations, and to ascertain in particular their relationship to Huxley's equation.

The simplest case, in which x and y are simple autocatalytic, or logistic, functions of time, has already been investigated by Bernstein ('34), whose work will be discussed more fully below. Two other equations have been commonly employed in the analysis of growth, namely the generalized autocatalytic and Gompertz functions. In the succeeding sections a detailed analysis of the relative growth functions derived from these three types of equations will be presented, with special reference to their relationship to (1). The investigation will be limited in extent to those cases in which the equations expressing the absolute growth of x and y are of the same type, although this is of course not necessarily true in general. This work was originally suggested by Dr. A. H. Hersh, to whom the author is deeply indebted for many helpful comments and suggestions.

THE SIMPLE AUTOCATALYTIC FUNCTION

In this case the growth-rates of x and y are given by the differential equations

$$\begin{cases} \dfrac{dy}{dt} = r_1 y(A_1 - y) \\ \dfrac{dx}{dt} = r_2 x(A_2 - x), \end{cases}$$

the integrated forms of which are

$$\begin{cases} \log \dfrac{y}{A_1 - y} = K_1(t - t_1) \\ \log \dfrac{x}{A_2 - x} = K_2(t - t_2). \end{cases}$$

A_1 and A_2 are the upper asymptotes of the curves, $K_1 = r_1 A_1$, and $K_2 = r_2 A_2$, while t_1 and t_2 are the abscissae of the points of inflection. Solving each of these equations for t and equating the solutions, we have

$$t = \frac{1}{K_1} \log \frac{y}{A_1 - y} + t_1 = \frac{1}{K_2} \log \frac{x}{A_2 - x} + t_2$$

from which is obtained the expression

$$\frac{y}{A_1 - y} = \frac{1}{C}\left(\frac{x}{A_2 - x}\right)^k \tag{2}$$

where $C = e^{-K_1(t_2 - t_1)}$ and $k = K_1/K_2$. Or, solving for y, we have

$$y = \frac{A_1}{C\left(\frac{A_2 - x}{x}\right)^k + 1}. \tag{3}$$

From equation (1) it follows that

$$\frac{dy/y}{dx/x} = k.$$

Considerable biological significance has been attached to k, which has been termed by Robb ('29) and Huxley ('32) the coefficient of growth-partition, as representing the ratio of the percentage growth-rates of x and y. Equations (1) and (3) may therefore best be compared by investigation of the behavior of $(dy/y)/(dx/x)$ for the latter, after the manner of Bernstein ('34). For convenience, the notation $R = (dy/y)/(dx/x)$ will be used.

Differentiating (3) and multiplying the derivative by x/y, we obtain

$$R = \frac{A_2 C k (A_2 - x)^{k-1}}{C(A_2 - x)^k + x^k}. \tag{4}$$

From this equation it is readily seen that

$$\lim_{x \to 0} R = k = \frac{r_1 A_1}{r_2 A_2}.$$

Thus an approximation to (1) is realized for small values of x and y. This is evident also from (2), for when x and y are small they become negligible in comparison to their final values A_1 and A_2. We may then write as a first approximation

$$\frac{y}{A_1} = \frac{1}{C}\left(\frac{x}{A_2}\right)^k, \qquad \text{or} \qquad y = \frac{A_1}{C A_2{}^k} x^k,$$

which is obviously of the form of (1). It should be noted, however, that k, as here derived, is not simply the ratio of the velocity constants r_1 and r_2, but depends also on A_1 and A_2. Likewise, b involves here the ratio of the final, not the initial values of x and y.

If we examine the behavior of R as x and y approach their final values, we find that

$$\lim_{x \to A_2} R = 0, \quad C, \quad \text{or} \quad \infty$$

as $k \gtrless 1$. The graph of equation (3) obtained by plotting log y against log x is thus a smooth curve which becomes approximately rectilinear toward the lower end, but whose slope, when $k \neq 1$, approaches toward the upper end a final value independent of the precise value of k. When $k=1$, the final value of R, namely C, depends primarily on the difference between t_2 and t_1. In particular, if $t_2=t_1$ then $C=1$, and (3) becomes linear. Curves obtained for different values of k are shown in Figure 1.

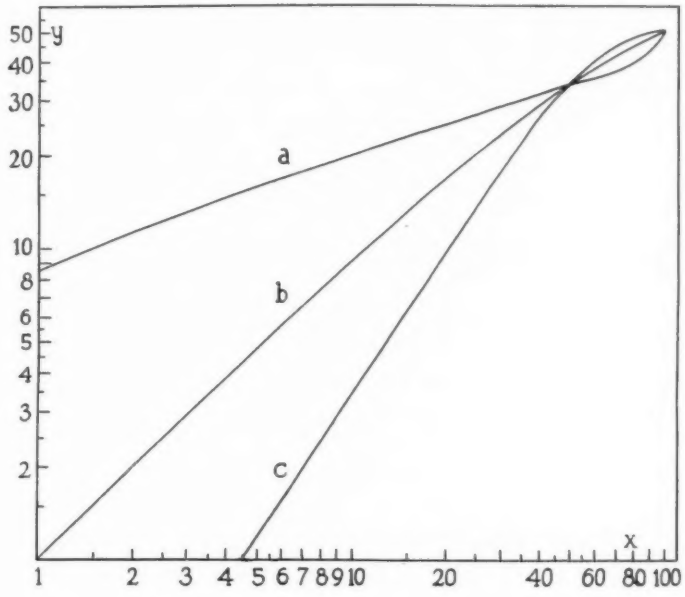

FIG. 1. Double logarithmic graph of equation (3) for $A_1=50$, $A_2=100$, $C=0.5$, and (a) $k=0.5$, (b) $k=1$, (c) $k=1.5$, to show the form of the relative growth curves when the time course of growth of x and y follows the simple autocatalytic curve.

The above conclusions are essentially similar to those of Bernstein ('34), who uses a somewhat different form of the logistic function from that employed here. Bernstein states, however, that when $k=1$ the relative growth function is in all cases linear. That this statement is incorrect is evident upon inspection of his equation (p. 212), which, for $k=1$, behaves in essentially the same fashion as equation (3).

Investigation of the variation of R over the interval $0<x<A_2$ discloses the further important fact that, except for $k=1$, R is not a monotonic function of x, but has one maximum or minimum within the interval. This can be demonstrated by the usual procedure of calculating dR/dx and equating it to zero, which in this case yields

$$C + (1 - k)\left(\frac{x}{A_2 - x}\right)^k - k\left(\frac{x}{A_2 - x}\right)^{k-1} = 0.$$

If we make the substitution

$$\frac{x}{A_2 - x} = \frac{1}{z},$$

we have

$$C + \frac{1 - k}{z^k} - \frac{k}{z^{k-1}} = 0$$

or

$$Cz^k = k(1 + z) - 1 \tag{5}$$

in which the interval $0 < x < A$ is replaced by $z > 0$. If $k = 1$, (5) becomes linear with a root at $z = 0$, and consequently R is monotonic for $z > 0$. On the other hand, for any value of $k \neq 1$ it can be shown that (5) has one and only one positive root, say $z = z_0$, and that dR/dx changes sign in the neighborhood of z_0. From this it follows that R has an extreme value at this point, and the log-log plot of equation (3) is therefore a sigmoid curve with an inflection at the value of x corresponding to z_0. This value is a function of the various parameters involved, but the nature of the function can be determined only for specified values of k.

The sigmoid character of the relative growth curve, although analytically demonstrable, is not always apparent from the curve itself, due to the flattening of the latter toward its lower end. In fact, if the point of inflection occurs at a very small value of x, it may not be included in the curve at all. Thus in curve (a) of Figure 1, a slight sigmoid trend is visible; in curve (c), on the other hand, there is no apparent inflection.

THE GENERALIZED AUTOCATALYTIC FUNCTION

This function is derived from the simple autocatalytic by the introduction of an additional constant to give the curve a lower asymptote different from zero. The differential equations expressing the growth-rates of x and y become

$$\begin{cases} \dfrac{dy}{dt} = r_1(y - B_1)(A_1 - y) \\ \dfrac{dx}{dt} = r_2(x - B_2)(A_2 - x). \end{cases}$$

Integration of these gives

$$\begin{cases} \log \dfrac{y - B_1}{A_1 - y} = K_1(t - t_1) \\ \log \dfrac{x - B_2}{A_2 - x} = K_2(t - t_2) \end{cases}$$

where $K_1=r_1(A_1-B_1)$, $K_2=r_2(A_2-B_2)$, and t_1 and t_2 have the same significance as in the preceding section.* The elimination of t between these equations yields the relationship

$$\frac{y-B_1}{A_1-y}=\frac{1}{C}\left(\frac{x-B_2}{A_2-x}\right)^k \tag{6}$$

or

$$y=\frac{A_1(x-B_2)^k+B_1C(A_2-x)^k}{(x-B_2)^k+C(A_2-x)^k} \tag{7}$$

where $C=e^{-K_1(t_2-t_1)}$ and $k=K_1/K_2$. If B_1 and B_2 are both zero, (6) and (7) reduce to (2) and (3) respectively.

(6) may be written in the form

$$\frac{y-B_1}{A_1}\cdot\frac{1}{1-\dfrac{y}{A_1}}=\frac{1}{C}\left(\frac{x-B_2}{A_2}\right)^k\left(\frac{1}{1-\dfrac{x}{A_2}}\right)^k.$$

As x and y approach zero, the last factor on each side of this equation approaches unity. Hence, for small values of x and y, we may write as a first approximation

$$\frac{y-B_1}{A_1}=\frac{1}{C}\left(\frac{x-B_2}{A_2}\right)^k$$

or

$$y-B_1=\frac{A_1}{CA_2{}^k}(x-B_2)^k. \tag{8}$$

This approximation has little value, of course, unless B_1 and B_2 are negative, or at most small positive numbers. Equation (8) is not linear in log x and log y, and reduces to (1) only when B_1 and B_2 are both zero.

The behavior of (7) is somewhat complicated, due to the presence of variable lower asymptotes for x and y. We have

$$R=\frac{kCx(A_1-B_1)(A_2-B_2)(x-B_2)^{k-1}(A_2-x)^{k-1}}{[(x-B_2)^k+C(A_2-x)^k][A_1(x-B_2)^k+B_1C(A_2-x)^k]}. \tag{9}$$

When B_1 and B_2 are positive, it is readily seen that

$$\lim_{x\to B_1}R=0,\quad \frac{B_2(A_1-B_1)}{CB_1(A_2-B_2)},\quad \text{or}\quad \infty$$

* Titus ('30) has shown that the differential equation of the generalized autocatalytic function is identical in form with that of Crozier's equation (Crozier, '26). The integrated forms, however, are not identical, for the graph of Crozier's equation always passes through the origin, which is not true of the generalized autocatalytic. Hence the latter is a less restricted form than the former.

as $k \gtrless 1$. When B_1, B_2, or both, are negative, a number of situations arise, which may be summed up in the following two; either $y \geqq 0$ when $x=0$, or $x=x_1>0$ when $y=0$. In the former case we have

$$\lim_{x \to 0} R = 0.$$

In the latter we have

$$\lim_{x \to x_1} R = R_1$$

where R_1 is finite and positive.

At the upper end of the curve it is found that

$$\lim_{x \to A_1} R = 0, \quad \frac{CA_2(A_1 - B_1)}{A_1(A_2 - B_2)}, \quad \text{or} \quad \infty$$

as $k \gtrless 1$. It is thus evident that several types of curves may arise, which can be classified as follows:

1. B_1 and B_2 are positive.
   (a) $k>1$. R varies from 0 to 0.
   (b) $k<1$. R varies from ∞ to ∞.
   (c) $k=1$. R varies from $B_2(A_1-B_1)/CB_1(A_2-B_2)$ to $CA_2(A_1-B_1)/A_1(A_2-B_2)$.
2. B_2 or both B_1 and B_2 are negative. $y \geqq 0$ when $x=0$.
   (a) $k>1$. R varies from 0 to 0.
   (b) $k<1$. R varies from 0 to ∞.
   (c) $k=1$. R varies from 0 to $CA_2(A_1-B_1)/A_1(A_2-B_2)$.
3. B_1 or both B_1 and B_2 are negative. $x=x_1>0$ when $y=0$.
   (a) $k>1$. R varies from R_1 to 0.
   (b) $k<1$. R varies from R_1 to ∞.
   (c) $k=1$. R varies from R_1 to $CA_2(A_1-B_1)/A_1(A_2-B_2)$.

Further analysis of the behavior of R by the method employed in the preceding section is unfeasible here, due to the alegebraic complications which arise. It is apparent, however, that curves of types 1a, 1b, and 2a must have at least one point of inflection. In curves of the remaining types, an inflection may or may not appear, depending on the relative values of the parameters A_1, A_2, B_1, B_2, C, and k. This can be verified by plotting several curves for various arbitrarily selected values of these parameters. Without further investigation nothing can be said regarding the position of the point of inflection or the possible existence of more than one inflection. Examples of the different types of curves obtained are shown in Figures 2 and 3. It should be noted that if x is taken as the size of the whole organism, curves of type 2 can have no biological significance, for this would mean that the size of the part y has a positive value when that of the organism is zero.

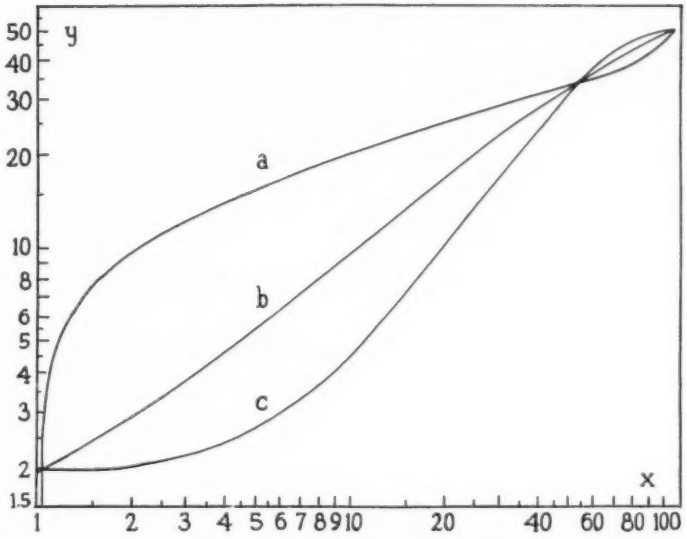

FIG. 2. Double logarithmic graph of equation (7) for $B_1=2$, $B_2=1$, and the remaining constants as in Figure 1. This shows the form of the relative growth curves when the time course of growth of x and y follows the generalized autocatalytic curve with the lower asymptote in each case positive (type 1, p. 146).

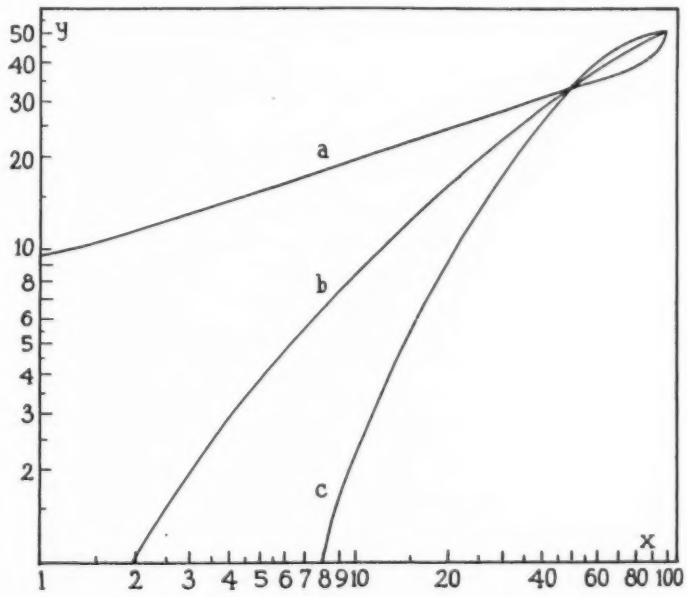

FIG. 3. Same as Figure 2, except that $B_1=-2$, $B_2=-1$; i.e., the lower asymptotes are both negative Curve (a) is of type 2b, while (b) and (c) are of type (3) (see p. 146).

THE GOMPERTZ FUNCTION

This equation has been derived on the assumption that the percentage growth-rate falls off exponentially with time (see Weymouth and McMillin, '30; also Winsor, '32). The percentage growth-rates of x and y may therefore be written

$$\begin{cases} \dfrac{dy}{ydt} = a_1 e^{-r_1 t} \\ \dfrac{dx}{xdt} = a_2 e^{-r_2 t}. \end{cases}$$

Integration of these gives

$$\begin{cases} \log y = -c_1 e^{-r_1 t} + b_1 \\ \log x = -c_2 e^{-r_2 t} + b_2 \end{cases}$$

where $c_1 = a_1/r_1$, $c_2 = a_2/r_2$, and b_1 and b_2 are constants of integration. Or

$$\begin{cases} y = A_1 e^{-c_1 e^{-r_1 t}} \\ x = A_2 e^{-c_2 e^{-r_2 t}} \end{cases}$$

where $A_1 = e^{b_1}$ and $A_2 = e^{b_2}$. Solving each of these for t and equating the solutions we have

$$t = -\frac{1}{r_1}\left(\log\log\frac{A_1}{y} - \log c_1\right) = -\frac{1}{r_2}\left(\log\log\frac{A_2}{x} - \log c_2\right)$$

$$\log\log\frac{A_1}{y} = \log\left(\log\frac{A_2}{x}\right)^k + \log K$$

where $k = r_1/r_2$ and $K = c_1/c_2^k$. When solved for y, this expression becomes

$$y = A_1 e^{-K\left(\log\frac{A_2}{x}\right)^k}. \tag{10}$$

When $k = 1$, the above equation reduces to

$$y = \frac{A_1}{A_2{}^K}\, x^K$$

which is clearly identical with (1). It should be observed, however, that since $r_1 = r_2$, we have $K = a_1/a_2$. The value of K thus depends simply on the ratio of two constants which affect only the lateral translation of their respective growth curves, and does not involve directly the velocity constants r_1 and r_2. The part y may therefore be growing more or less rapidly than x in a given time interval, although the rate of decrease of percentage growth is the same for both.

From (10) we obtain

$$R = Kk\left(\log\frac{A_2}{x}\right)^{k-1}. \tag{11}$$

When $K \neq 1$, it is evident that

$$\lim_{x \to 0} R = \infty \quad \text{or} \quad 0$$

and

$$\lim_{x \to A_2} R = 0 \quad \text{or} \quad \infty$$

as $k \gtrless 1$. Hence the graph of log y against log x is a smooth curve whose slope approaches, toward *either* end of the interval $0 < x < A_2$ a limiting value inde-

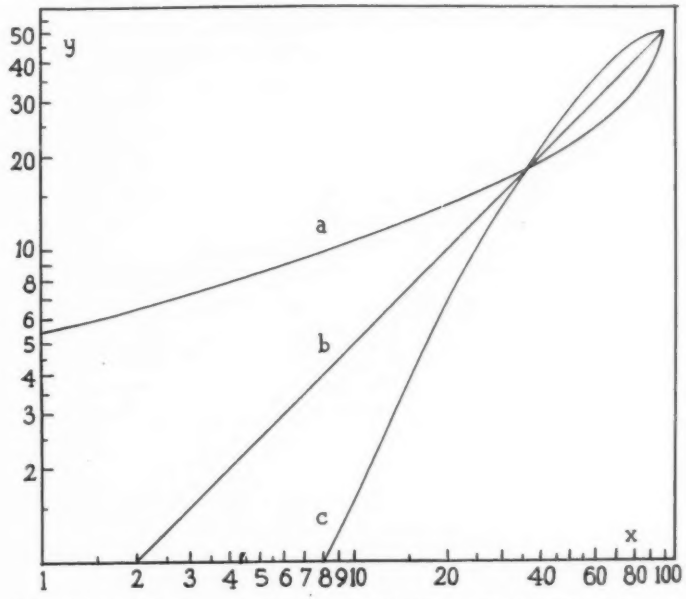

FIG. 4. Double logarithmic graph of equation (10) with $A_1 = 50$, $A_2 = 100$, $K = 1$, and (a) $k = 0.5$, (b) $k = 1$, (c) $k = 1.5$, showing the form of the relative growth curves when the time course of growth of x and y follows the Gompertz curve.

pendent of the precise value of k. When k is close to one, the curve flattens considerably in the middle portion of the interval, thus approximating equation (1). This approximation differs, however, from that obtained from equation (2) above, in that the latter may in all cases be extrapolated as far as we please toward $x = 0$, whereas the former may be so extrapolated only to a limited extent, dependent on the value of k.

It is easily shown that within the interval $0 < x < A_2$, R is monotonic. Differentiating (11), we have

$$\frac{dR}{dx} = \frac{Kk(1-k)}{x}\left(\log \frac{A_2}{x}\right)^{k-2}.$$

The right member of this equation cannot vanish for any value of x within the interval except when $k=1$, in which case it becomes identically zero. Hence the log-log plot of (10) can have no inflection, in which respect it differs from the corresponding graphs of (3) and (7). Representative curves for different values of k are given in Figure 4.

DISCUSSION

The derivation of the relative growth functions discussed in the preceding sections involves the general assumption that the growth of each part is determinate, i.e., that the size of the part approaches asymptotically a finite upper limit. Correspondingly, we find that these functions possess a common property, namely their peculiar behavior as x and y approach their final values. With respect to this property Bernstein ('34), in the case investigated by him, reaches the following conclusions: "This, of course, proves that percentage growth at the end of the growth process is completely absent. But it shows also that the percentage growth cannot characterize a ratio of partition during the whole process of growth of an organ of limited size." (p. 212).

These conclusions certainly apply to all the cases under present consideration, and it appears that in general, when the growth of the parts is determinate, the concept of a constant coefficient of growth partition, in the sense of Robb and Huxley, is not valid over the entire growth period. Moreover, even when an approximation to Huxley's equation is realized, the exponent k cannot be regarded as being simply a ratio of velocity constants, since it may also involve other parameters, as has been pointed out above. In fact, in the case of equation (10), Huxley's equation is obtained only when the constants characterizing the percentage growth-rates of x and y are equal.

We have seen that equation (1) is a first approximation to (3) for sufficiently small values of x and y. This arises from the fact the simple autocatalytic function itself approximates the exponential for small values of the independent variable. Since growth curves are frequently found to be approximately exponential in the early stages of the growth process, it is not surprising that Huxley's equation is widely applicable. Within the limits of the error involved in measurement and in the graphical methods usually employed, a close fit to a straight line may be obtained over a large portion of the growth period. However, if the data include values of x and y sufficiently close to their final values the curve must be expected to deviate from a straight line at its upper end.

Such deviations have been found to occur in a number of instances. The curves obtained by Huxley ('32, p. 58) for the mandible length/body length relationship in the males of three species of stag-bettles show a distinct resemblance to curve (c) in Figure 1. The same is true of the curves found by Paulian ('35) to express the relative growth of length of cephalic horn and elytron length in the beetles *Eurytrachelus reichii* and *E. bucephalus*. It is true that these are special cases, complicated by the presence of holometabolous development and

by the statistical character of the data. But similar curves are obtained also in cases not thus complicated, e.g., for the height/length relationship of the carapace in Daphnia pulex (L. J. Zupancic, unpublished data). In addition, many of those relative growth curves which exhibit breaks toward the end of the growth period probably belong in this category. Although such breaks may in many cases represent underlying biological changes, it is very likely that they are often of the purely illusory type which arises as a result of fitting straight lines to what is in reality a smooth curve (see for example Huxley, '32, fig. 55, p. 98). Certainly, considerable caution must be exercised in attempting to assign biological significance to breaks occurring in any particular case.

It has been shown that equation (7) has as a first approximation, for small values of x and y, the parabolic function

$$y - B_1 = b(x - B_2)^k \tag{8'}$$

which differs from Huxley's equation only by the presence of the parameters B_1 and B_2. The graph of this function on a double logarithmic grid is a smooth curve which approaches asymptotically a straight line with slope k as x and y increase indefinitely. Relative growth curves of this type sometimes occur in practice. Robb ('29) proposes for such curves a modification of (1) of the form

$$y = bx^k + c. \tag{12}$$

This expression is obviously identical with (8') if in the latter we set $B_2=0$. A similar flattening of the curve occurs in the case of equation (10) when k is close to one. It is evident from both (7) and (10), however, that (12) is not a valid approximation over the entire range of sigmoid growth, for as x and y approach their final values the curvature must again increase.

Robb regards the constant c in (12) as a correction representing the composite value of the amounts of inert (non-growing) substances in the primordia of x and y. If such corrections are introduced separately into the absolute growth functions expressing the sizes of x and y, it is seen that this interpretation is open to criticism. To take a simple case, suppose we have

$$\begin{cases} y - c_1 = a_1 e^{r_1 t} \\ x - c_2 = a_2 e^{r_2 t}. \end{cases}$$

The resulting relative growth function is

$$y - c_1 = b(x - c_2)^k.$$

In this equation c_1 and c_2 appear as discrete entities, which cannot be combined into a composite constant. From this standpoint c can at most be said to represent a correction for inert components of only the primordium of y.

There are several cases in which relative growth curves have been found to be sigmoid; these are of course explicable in terms of equations (3) and (7). When the presence of an inflection is accompanied by flattening of the curve, however, it may pass unnoticed because of the scatter of the observed points or be regarded as a random fluctuation. Thus the points of the graph of log body

weight against log body length in the mouse (Green and Fekete, '33) exhibit a conspicuous sigmoid trend, although the authors have fitted them by a straight line. In other cases the sigmoid character of the curve is more distinct. For the relationship between size of nipper claw and carapace length in the pistol crab, *Alpheus dentipes*, Dawes and Huxley ('34) obtained a smooth curve with a marked inflection. Sigmoid curves are likewise obtained for the leg length/body length and femur length/rest-of-leg length relations in Notonecta sp. (L. B. Clark, unpublished data). The relative growth of width and length in the fruit of *Capsicum annuum*, investigated by Kaiser ('35), furnishes an unusually interesting example. Kaiser finds a genetic difference in the form of the relative growth curve, due apparently to a difference in a single gene. The curve is sigmoid in one variety and approximately linear in the other, the sigmoid being dominant. The last two examples cited are especially significant in that the curves represent single specimens.

In addition to the cases discussed in the preceding paragraphs there are others in which the relative growth curve neither approximates a straight line over any appreciable interval nor exhibits a point of inflection (see Figures 3 and 4). Little can be said about any given curve of this type unless something is known of the absolute growth of the parts concerned. Such information is available in only a few cases. For example, Weymouth and McMillin ('30) find that growth in shell length of the Pacific razor clam, Siliqua patula, conforms well to the Gompertz function. It seems probable that this is true also of growth in shell width. With reference to the relative growth of width and length they state that k varies from 0.6 to about 1.2. It is evident from equation (10), which apparently applies in this instance, that no significance can be attached to these end values (see above, p. 149).

Inasmuch as certain types of deviations from Huxley's equation are to be expected as a natural consequence of sigmoid growth, there is in general no need for special hypotheses, such as have been advanced by some investigators, to account for each particular case. For example, Dawes and Huxley ('34) find that for the relative growth of crusher claw size and carapace length in Alpheus dentipes the coefficient k decreases monotonically with increasing body size. They suggest that this may be due to the physiological impossibility of maintaining the initial high value of the coefficient. In the light of the present analysis this explanation seems rather artificial and at the same time superfluous. A physiological explanation of the phenomena of relative growth is undoubtedly desirable, but it is clear that in seeking such an explanation we must regard these "deviations" as a general phenomenon resulting from the determinate character of the growth process, rather than as a series of isolated exceptions. The necessity of taking this into account will be apparent also from the considerations which follow.

The coefficient k in (1) and (12) is regarded by Robb ('29) as representing the ratio of partition of some growth-controlling substance between two parts of an organism, the partitioning occurring in a manner analogous to the distribution of a solute between two immiscible solvents when the quantity of

solute is insufficient to saturate both of them. This involves the assumption that the growth exhibited by a part is proportional to the quantity of growth-controlling substance available to it. Also, Teissier ('34) has shown that equation (1) is deducible if it is assumed that, as a first approximation, the quantity of food consumed by an organ in a given time interval is proportional to the size of the organ and to the total amount of food consumed within the same interval, also that a fixed fraction of the food is used for growth.

This last assumption is equivalent to that involved in Robb's theory, for both imply that $dx = cdm$ where x is the size of the organ, m the quantity of available materials for growth, and c a constant. Although this proportionality may hold over a short time interval, it is evident that least in the later stages of sigmoid growth the fraction of food used for growth must progressively decrease. Hence the theories of both Robb and Teissier are not valid for the entire growth process.

In this connection Bernstein ('34) remarks that "the idea of partition of food cannot be combined with the idea of multiplicative growth because limitation of food leads to the abandonment of the multiplicative scheme of growth" (p. 213). This means, of course, that the idea of food partition can hold only in the case of determinate growth, and that Robb's interpretation of the coefficient k is not entirely sound.

The considerations set forth in this section seem to warrant the general conclusion that while Huxley's equation may represent a useful first approximation to some more precise law of relative growth, it does not furnish an adequate basis for investigation of the underlying mechanism since it fails to take into account certain features of determinate growth. The method of approach which has been used in the present investigation serves to clarify some of these features, but is of limited value for further analysis of relative growth. In the first place, it is applicable only in the case of absolute growth functions which can be solved for t. Furthermore, the equations so obtained are apt to be complicated and their application to experimental data difficult, even when the functions from which they are derived are relatively simple. At the same time there is at present no satisfactory theoretical basis for determining what type of equation applies in any given case. The selection of an appropriate function for describing a particular set of data thus requires a knowledge of the nature of the absolute growth functions involved. In view of these circumstances, it seems that further progress in the investigation of the mechanism of relative growth must depend in large measure on the development of a different theoretical approach. At the present time there is also a need for more intensive experimental data, which will include, among other things, the time relations of the sizes of various parts of an organism.

SUMMARY

1. Functions expressing the relative growth of two parts of an organism are derived on the assumption that the growth curve of each part is sigmoid, con-

forming with a) the simple autocatalytic, b) the generalized autocatalytic, or c) the Gompertz function.

2. In each of these three cases a first approximation is given by a parabolic function, which in cases a) and c) takes the form of Huxley's equation. The approximation is not in general valid over the entire growth period.

3. In cases a) and b) the curve obtained on a log-log graph may exhibit a point of inflection; in c) it is found that no inflection can occur.

4. It is shown that certain types of deviations from Huxley's equation which occur in practice are explicable in terms of the relative growth functions derived here.

5. The idea of food partition in connection with relative growth cannot be based on Huxley's equation, since the latter fails to take into account certain characteristics of determinate growth.

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WHAT IS A GROWTH CYCLE?

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SUMMARY

This article is the first of a series dealing with the description of growth phenomena. Existing definitions of cycles of growth are reviewed. A more adequate definition is presented. The law of cyclic growth is stated and illustrated. Possible objections are considered and potential uses and values of the new definition are indicated.

THE NEED FOR DEFINITION

Increase in precision of definition is a symptom of progress in any science. Metaphysical alchemy changed to modern chemistry when the researches of Lavoisier, Priestly, and others made possible a more precise definition of the basic concept, "element." Similar illustrations from other fields will readily occur to all familiar with the history of science.

The biological sciences, however, seem to suffer from some pernicious limitation in definition which inhibits the discovery of *mathematical* relationships between forces and effects; relationships, that is, like those in the exact sciences where a high degree of prediction and control has been achieved. The limitation, according to Karl Pearson (1892), is that:

> the biologist looks to force, chemical constitution, molecular structure for an *explanation* where at best they can merely provide conceptual shorthand for descriptive purposes.

Certainly no one can read Hammett's ('35) intriguing array of facts, theories, hypotheses, opinions, assumptions, and surmises concerning the nature of growth without *feeling the need* for a certain something in the way of exact definition that biology has not yet achieved.

THE MEANING OF CYCLE

For example, consider the term "cycle" which occurs frequently in biological literature. The word is not the exclusive property of biology—it is, in fact, a borrowed word. Its general meaning, as given by Webster, is:

> One of the intervals, or spaces of time, in which one course, or round, of a certain regularly and continually recurring succession of events, or phenomena, is completed.

One finds "cycles" in mechanics, in thermodynamics, in electrodynamics, in mathematics, in history, in literature, in sociology and in many other fields. In biology there is a similar multiplicity of cycles; specific cycles, such as the carbon cycle, the cardiac cycle, etc., and more general cycles, such as life cycles,

reproductive cycles, evolutionary cycles, etc. Last, but not least, there are "growth" cycles, the subject of this paper.

The use of the term, cycle, with suitable modifiers is quite appropriate in biology. For much of life consists of rounds of events which return upon themselves, as it were, restoring the original states from which the cycles of events began. But when an organism "grows," there is only progress forward. The individual passes through a series of related phases of developments, to be sure, but in no sense is the growth series circular in character, *except as the various phases are repeated in the life of another individual.* In the basic sense, cycle and growth might seem to be antithetical terms.

In other fields, however, the connotation of cycle has been extended to mean an age or period of time during which certain types of events occur; as the "colonization" period, or "cycle," in the history of a nation. The events in the rise and fall of nations are cyclic only in the sense that they, too, are repeated over and over again in the lives of different nations. Therefore, it really is appropriate to couple growth and cycles to mean a succession of periods, or pulses, or waves of growth. In growth cycles, it should be noted, the phenomena that are "recurrent" are not phases of development in the organism, *but the phases in the process of growth itself.*

EXISTING DEFINITIONS OF A GROWTH CYCLE

In growth curves from certain types of situations gross fluctuations in measured values tend either to obscure the movement of growth, or to make it impossible to determine trends with certainty. However, it is common experience in scientific research that

(1) the simpler the situation;
(2) the greater the constancy of conditions; and
(3) the greater the reliability of the measurements;

the easier it is to discern and determine any quantitative relationship that may be present. It seems permissible, therefore, to select for analytical study growth curves from situations known to be fairly stable.

Mere casual inspection of growth curves from many stable situations yields at once the impression of distinct cycles of growth. This fact has been pointed out by many investigators (Figure 1).

Backman ('31) ascribes to Robertson ('08) the introduction of the concept of "growth cycles," but Robertson, Backman, and others who have made use of the concept have merely defined it in general terms and have not established adequate criteria for determining whether one is dealing with a cycle or with mere chance variations in measurements.

For instance Backman ('31), after criticizing severely Robertson, Brody, Schmalhausen, Saller and others who assume cycles at will, quotes Fauré-Fremiet's* definition of cycle as identical with his own.

* "Am besten und klarsten und mit meiner Definition identish hat schon 1925 Fauré-Fremiet den Wachstumszyklus definiert: 'Nous pouvons donc dire que la caractéristique d'un cycle est que, au cours

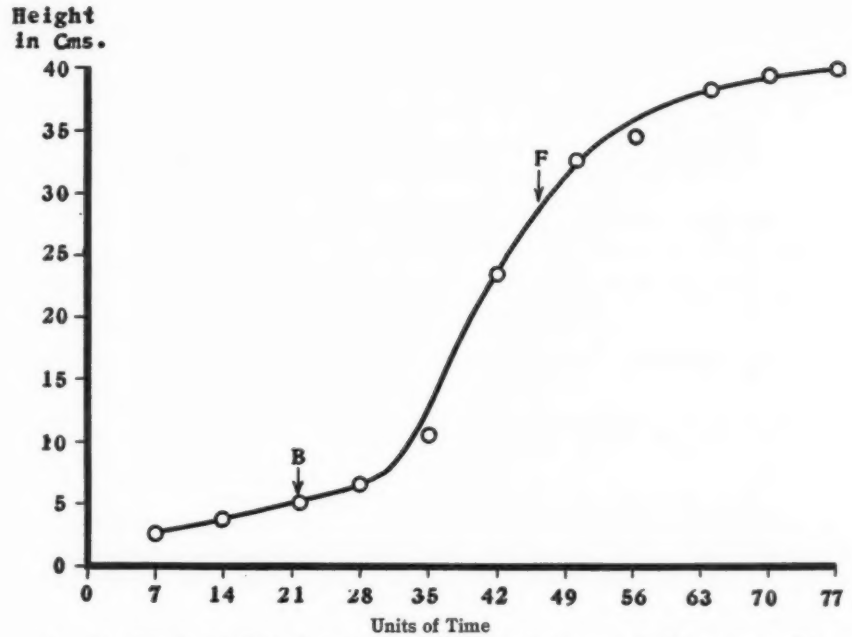

FIG. 1A. Growth of individual plant No. 428, Impatiens Balsamae, under laboratory conditions; eight-hour photo-period, height measured from surface of soil to terminal bud. B indicates time when flower buds were first observed; F indicates time of first opening of flower buds.

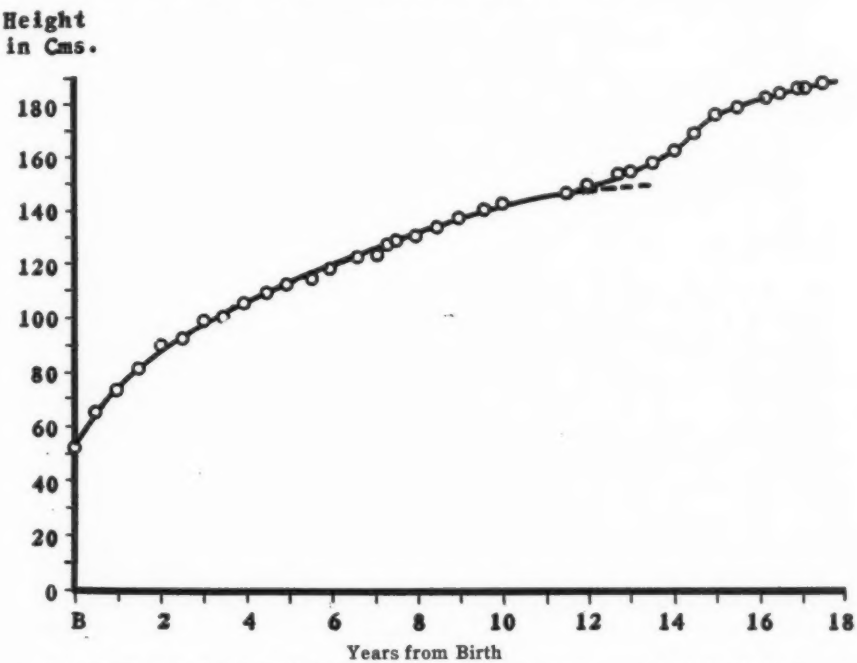

FIG. 1B. Growth of an individual boy in height. Based on measurements made by the boy's father practically every half-year from birth to maturity.

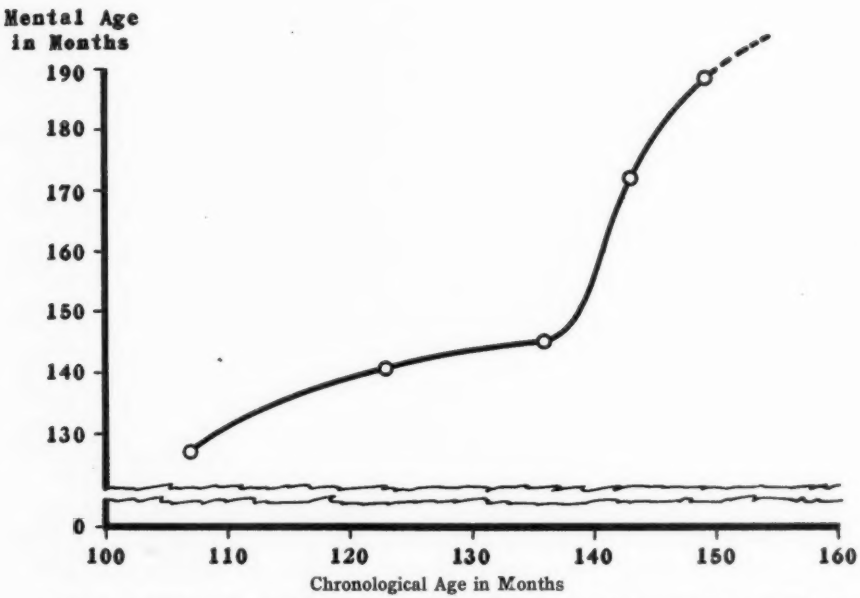

FIG. 1C. Growth of an individual in score on an intelligence test. The curve presents graphically the typical change in the relation of mental age to chronological age as the individual passes from childhood into adolescence.

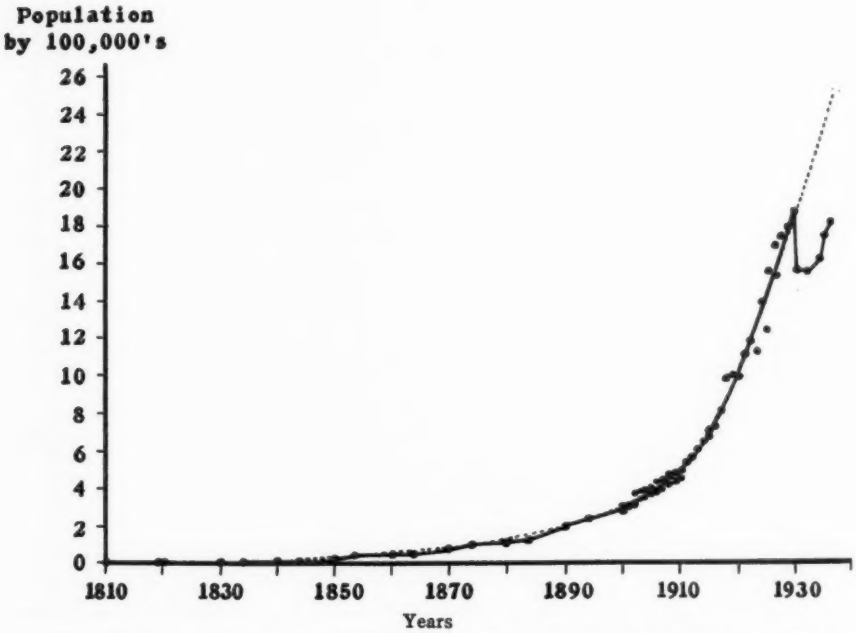

FIG. 1D. Growth of an individual city in population. Based on population figures for the City of Detroit as given in the city directory. For certain years two and sometimes three estimates of population are given. Each of these is represented by a dot in the figure to give some idea of the unreliability of the data.

father practically every half-year from birth to maturity.

We can say, therefore, that the characteristic of a cycle is that in its course the rate* of growth varies continually, and passes through a maximum. The variations of this rate as a function of time are represented by a bell shaped curve.

Such a definition calls attention to the important fact that at certain periods growth follows a definite pattern. However, it does not describe that pattern in quantitative terms nor does it supply the fundamental criteria for determining the conditions under which growth cycles occur. Greater precision in definition is much needed.

It will be evident to anyone, from an inspection of the curves in Figure 1, that there is in each curve an *appearance* of two or more waves, or impulses, or periods, of growth, and that the general form of the growth curve during each of these periods resembles somewhat the well-known "S" or "autocatalytic" curve that is always found in whatever field growth is studied. As early as 1874, Sachs observed this similarity of form in different growth curves and formulated, as a definition of growth, the statement:

To describe an important fact briefly I shall call the incipient increase, the reaching of a maximum, and the final decrease in the growth rate of a portion of a plant, the great period of growth.†

The phrase, "the great period of growth," has found its way into biological literature, and is used to this day by many writers without much questioning of what the statement means. What Sachs, Backman, Fauré-Fremiet ('25) and others define as the great period of growth is nothing more than *growth during a given cycle*. Backman himself recognizes this fact when he says ('31, p. 911):

Let us then keep to the definition of Sachs and say that there are as many growth cycles as there are great periods. . . . ‡

CONDITIONS GIVING RISE TO GROWTH CYCLES

If, now, we ask ourselves "Under what circumstances may we expect to obtain that regularity of growth pattern which is the distinguishing characteristic of a great period?", it does not require deep reflection to arrive at the con-

de celui-ci, la vitesse de croissance varie continuellement et passe par un maximum. Les variations de cette vitesse en function de temps sont alors représentées par une courbe en cloche.' Das ist nichts anderes als eine unbewusste Wiederholung der von Sachs gegebenen Definition der grosse Periode, hier also das Charakteristische eines Wachstumszyklus hingestellt."

* Many biologists, both ancient and modern, analyze measurements of growth into the increments made during the successive units of time. Such analyses result in distributions, not curves. "Rate of growth" as used by Backman, is the specific (or relative) magnitude of the increase made during any unit of time. Hence the form in which his statement is cast.

The author, however, finds it simpler, mathematically, to deal with growth data as curves derived from measurements of the total amount of growth achieved at the end of the various units of time. Such a growth curve may also be derived from a distribution by accumulating the frequencies. See Table 1, columns 2, 2a, 3, and 3a, page 165.

† "Ich werde im folgenden, um eine wichtige Tatsache kurz bezeichnen zu können, die anfängliche Zunahme, Erreichung eines Maximums und endliche Abnahme der Wachstumsgeschwindigkeit eines Pflanzenteils, als die grosse Periode des Wachstums bezeichnen." (Quoted by Backman)

‡ 'Halten wir fest an der Definition von Sachs, und sagen wir also, es gibt so viele Wachstumszylen, wie 'Grosse Perioden' vorhanden sind."

data.

clusion that the pattern of growth will be most clearly revealed when growth takes place undistorted by changes in the factors acting.

Growth is a complex and dynamic process of change. There is always an immature organism which changes as it grows, and there are always forces acting to bring about the changes. As the organism matures it passes through a series of distinctive changes but eventually arrives at a mature or stable state or condition. Moreover, each part or phase of growth passes through a similar

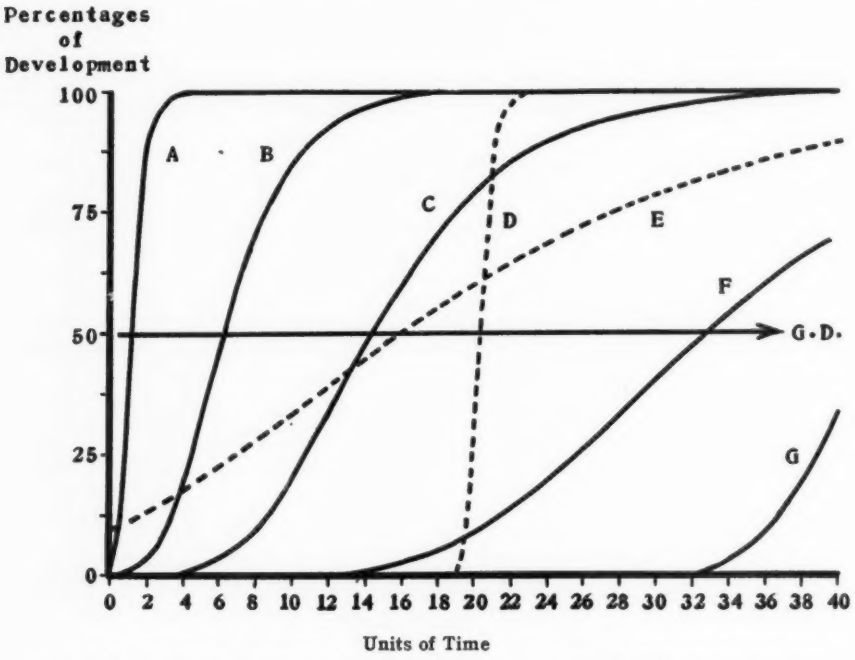

FIG. 2. Diagrammatic representation of the relation between specific maturations and general maturation.

Curves A, B, C, etc., represent specific maturation curves. The arrow indicates the progress of general maturation as the resultant effect of the specific maturations.

maturation process. In the cutting of a particular tooth, or the learning of the spelling of a given word, for instance, there is a time when the tooth is not cut, the spelling unknown. As the growth process proceeds, the point of the tooth appears through the gums, the word is spelled, but incorrectly. The child may be said to be mature with respect to these two *specific* growths when the tooth is completely cut, and the spelling of the word completely mastered. In each instance *the measurement of the progress of maturation yields an "S" curve.*

The maturation of an individual as a whole may be conceived as a complex process in which there are a whole series of small, or partial, growth processes

similar to cutting teeth, or learning to spell words, each of which *in itself* follows a definite pattern. Some of these maturations take a longer period of time than others, and they proceed, sometimes independently and sometimes *interdependently*, but each, whether large or small, whether the growth of a part, or the growth of a whole, can be studied *under constant conditions** and the various patterns of growth compared. (Figure 2.)

The emphasis on "constant conditions" is warranted because the idea of studying the growth process *independently* of the changes produced in it by changes in the factors acting is apparently novel in biological analysis. Almost all published studies of growth deal only with the gross phenomena of change in the growing organism. Yet upon reflection, one can readily see that a miscellaneous sampling of gross changes, caused *partly* by the growth process itself and *partly by changes in the factors involved*, cannot yield a clear picture of what happens *when an organism matures under constant conditions*.

It is well established by experimentation that it is possible to mutilate or otherwise change the organism *while it is growing*. It is also possible *to alter the character and intensity of the environmental forces acting*. All such changes in essential factors, however, immediately distort the pattern of growth. Such distortions are not part of the true growth pattern and tend to obscure it. Thus, our analysis of the growth situation yields *two* problems for study:

a. The problem of describing quantitatively the pattern of growth when all factors except growth are held constant; and
b. The problem of measuring the distortion of the growth pattern caused by changes in one or more of the factors acting.

This paper deals only with the first of the two problems.

PROPOSED IMPROVEMENTS IN DEFINITION

A "cycle of growth" occurs whenever four limiting conditions are present in the growth situation. There must be:

a. *Progress* toward a *specific* state or condition;
b. Made by an *immature organism* (or any unitary part, or process, of an organism);
c. Because the organism is acted upon by appropriate environmental forces; and
d. All the factors acting must remain *constant* throughout the period of maturation.

To the degree that the foregoing analysis is acepted, it is possible to define a growth cycle in a new and more useful way:

> *A growth cycle is any period in a specific maturation process during which all the effective factors remain constant.*

* Compare with Fauré-Fremiet's statement ('25): "Mais nous avons vu que le courbe générale exprimant un 'cycle de croissance' résulte de là superposition des courbes propres à chaque organe, à chaque tissu; nulle raison de s'arrêter ici, car cette dernière courbe exprime la somme algébrique des accroissements propres de chaque élément cellulaire du tissu on de l'organe considéré.

QUESTIONS

When such a concept of a growth cycle is considered, a number of questions come to mind. One is the problem of discriminating between increment which is developmental and increment which is not. Perhaps instead of calling the cycle defined above a "growth" cycle, it should be called a "maturation" cycle, because "growth" is a very general term. The distinction between growth and maturation will be considered at length in a later paper; the present article is concerned only with *that phase of growth* which corresponds to a "great period," or a "maturation" cycle.

Then there is the problem of the influence of one cycle of growth upon another. Does width of jaw mature of itself, or only as the advent of the permanent teeth stimulates the widening? This question and others of the same type can be answered only when *first* the characteristics of a simple cycle have been fully determined.

THE PATTERN OF CYCLIC GROWTH

Turning from all such problems—and there are many of them—let us consider instead what is the character of a specific instance of growth when neither the nature of the organism, nor any of the forces acting upon it, are altered *in the slightest* during the entire period of maturation.

One may well ask, "Why consider such an idealistic situation when one knows in advance that actual conditions will never remain constant?" The answer is that in science all the laws of nature—the law of gravitation, for instance—represent idealistic generalizations of behavior which the actual behavior studied approaches as a limit as control of conditions approaches perfection. No one will deny that the ideal laws in physics, chemistry and astronomy have contributed helpfully to prediction and control in those fields. It is probable, therefore, that similar generalizations of law in biology will prove equally efficacious in the study, analysis, and control of growth. However, perhaps a better form of statement of the problem would be, "Let us consider what pattern a *specific* maturation in a growing organism approaches as a limit, when both the *organism* and the *forces* acting upon it are held *as constant as possible* during a single and complete cycle of maturation."

Elsewhere the evidence has been presented that under standard and constant conditions, developmental growth takes place in such a fashion that the

> *increases in the logs of the percentages of development from a given point of reference are directly proportional to the increases in time, measured from the time of the point of reference.* (Courtis, '29, '32)

Expressed in mathematical symbolism, any biological growth under the defined conditions, whether of single organisms (as a cell), parts of organisms (as a tooth), or of a group of organisms (as a population), may, during a single cycle, be described very precisely by the formula*

* The formula for the pattern of growth during a single cycle was first reported by Benjamin Gompertz in 1825 according to the Philosophical Transactions of the Royal Society of London, England (page 513). The original form was

$$L_x = d \cdot g^{\neg g^x}$$

$$y = ki^{r^t}$$

in which "y" is the measured amount of development at time "t", "k" is the maximum toward which maturation progresses, "i" is the degree of development at the time chosen as the point from which to measure development,* and "r" is the rate of growth.

OBJECTIVE ILLUSTRATIONS

Perhaps the best way to objectify the foregoing mathematical description is to present the facts and figures for two simple- (or single-) cycle growth curves, and show the degree to which description by formula is exact. The specific maturation chosen as illustrations is the "attainment of marital status" by a defined group.†

The sex drive is one of the strongest in nature; nevertheless individuals differ not only in the strength of the drive but in their opportunities for satisfying it. The conditions of marriage in a given national culture are quite stable. If now we study the rate at which, in a given population, individuals attain the married state, we obtained a relatively pure single-cycle maturation curve.

Two curves for the attainment of the married state will be presented for comparison; one derived from data reported by Pearl ('25) for age of marriage of a small heterogeneous group of 242 men who came to Johns Hopkins for an operation for prostitis, the other derived from the statistics for age of marriage of Australian men from 1908 to 1932 (Commonwealth B. S. C. '32). The government tables of marriage statistics report *all* the marriages occurring each year,

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in which d, g, and q, are statistical constants, and x is the variable, time. However, experimental investigations have revealed what each of these constants stands for, and the original letters have been modified to serve as abbreviations.

The most general formula for the relationships between the elements involved in the description of growth is

$$y = ke^{ce^{at}}$$

in which e represents the incommensurable number, 2.71828+ and c and a are constants. The form of the resulting curve is determined by whether k, c, and a are given positive or negative, integral or fractional values. Altogether there are eight branches to the curve and between them they cover both increase and decrease, terminal and unlimited growth. The particular pattern dealt with in this article is but one of the family of eight growth curves, albeit the one most frequently observed in nature.

A very convenient formula for the standard pattern is

$$y = ke^{-e^{-r}}$$

where T represents the time required for the ordinate at the point of inflection to increase to its own power of itself.

* One cannot grow something from nothing. Because all life comes from pre-existing life, growth never starts from zero. Frequently, however, the movement of growth at its incipiency is below the threshold of measurable change. The symbol i, thus represents the degree of the development at the time arbitrarily chosen as the "beginning" of the cycle.

† Individuals who feel that the illustrations chosen are not representative of growth cycles because groups of individual men are not organisms in the conventional meaning of the term should remember two facts: (1) that even an individual organism is really a population of cells, and (2) that the form of the curves of these illustrations is precisely similar to those of the cycles of individual growth shown in Figure 1.

but to meet the criteria, *b* and *d* above only such numbers were taken from the tables as represented the marriages of *men born in a given year*. The birth year chosen was 1900 (to avoid as far as possible the distorting effects of the World War), and the total number of men ultimately married from this group was 41,000 a large group. The raw data are given in columns 2 and 2a of Table 1.

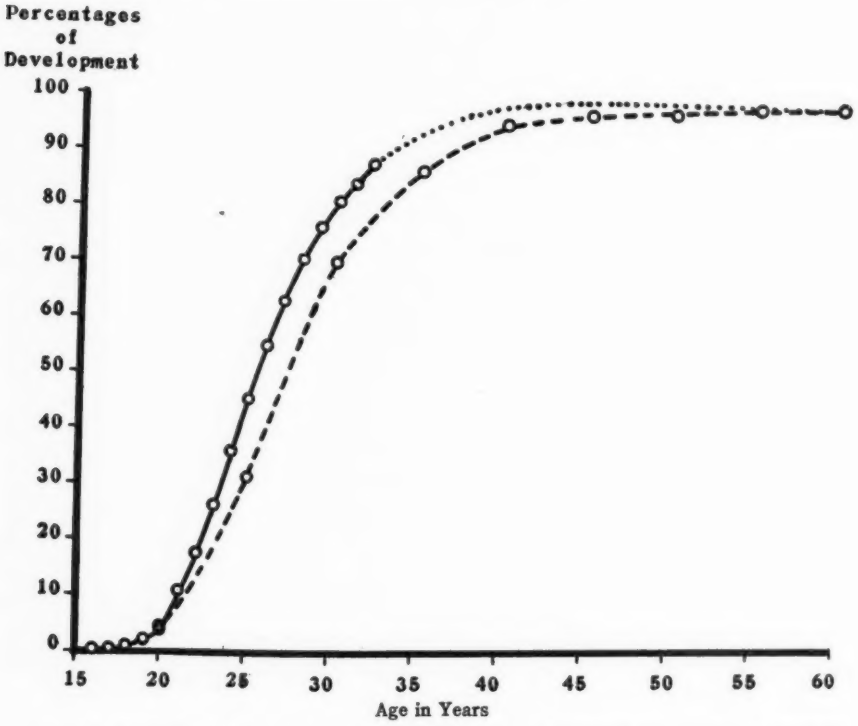

FIG. 3. Two curves for the attainment of marital status in men plotted as percentages of development to facilitate comparison.
Solid line—41,000 Australian men, all born in the year 1900
Broken line—242 heterogeneous American men

Maturation is a process of development ; it is not a matter of absolute magnitude. To compare the patterns of development of the two unequal groups, it is necessary to reduce the raw data to comparable terms. By dividing each set of accumulated frequencies by its respective total, all are reduced to comparable percentages of development. (Columns 4 and 4a, Table 1) Both sets of percentages of development were graphed in the same figure for comparison (Figure 3). The similarity in the forms of the two curves is at once apparent. The Australian men merely marry at a slightly higher rate than the American group.

TABLE 1

RELATION BETWEEN AGE AND THE ATTAINMENT OF MARITAL STATUS IN MEN

PART I

American Men. Total Number in Group 242

1	2	3	4	5	6	7	8	9	10
Age at Marriage	Number of Men Married	Accumulated Number Married	Per cent of Total Married	Logogs of Percentages	Differences from Point of Reference		Ratios		
					Time	Logogs	Time	Logogs	Per cent of Error
15	9								
20	67	9	3.72	0.15518	5	0.45355	1	0.98*	−2
25	94	76	31.40	−0.29837	0	0.0	0	0.0	0
30	40	170	70.25	−0.81432	5	0.51595	1	1.11	11
35	21	210	86.78	−1.21057	10	0.91220	2	1.97	−1
40	5	231	95.45	−1.69414	15	1.39577	3	3.02	0.6
45	2	236	97.52	−1.96224	20	1.66387	4	3.60	−10
50	3	238	98.35	−2.14137	25	1.84300	5	3.99	−20
55	1	241	99.59	−2.74958	30	2.45121	6	5.30	−12
60	0	242	100.00		35		7		

* Obtained by dividing 0.45355, the lolog difference between −0.29837 and +0.15518 by ±0.46233 the average five-year lolog difference between ages 20 and 40, or a yearly difference of ±0.092466.

PART II

*Australian Men. Total Number in Group 41,000**

1a	2a	3a	4a	5a	6a	7a	8a	9a	10a
Age at Marriage	Number of Men Married	Accumulated Number Married	Per cent of Total Married	Logogs of Percentages	Differences from Point of Reference		Ratios†		
					Time	Logogs	Time	Logogs	Per cent of Error
16	4	4	.01	0.60206	9	1.06648	9	9.30	3
17	42	46	.11	0.47109	8	0.93551	8	8.16	2
18	228	274	.67	0.33724	7	0.80166	7	6.99	−0.1
19	484	758	1.85	0.23879	6	0.70321	6	6.13	2
20	1020	1778	4.34	0.13434	5	0.59876	5	5.22	4‡
21	2557	4335	10.57	−0.01062	4	0.45380	4	3.96	−1
22	2933	7268	17.73	−0.12418	3	0.34024	3	2.97	−1
23	3473	10741	26.20	−0.23530	2	0.22912	2	2.00	0
24	4025	14766	36.01	−0.35304	1	0.11138	1	0.97	−3
25	3835	18601	45.37	−0.46442	0	0.0	0	0.00	0
26	3849	22450	54.76	−0.58247	1	0.11805	1	1.03	3
27	3487	25937	63.26	−0.70143	2	0.23701	2	2.07	3
28	2936	28873	70.42	−0.81734	3	0.35392	3	3.09	3
29	2467	31340	76.44	−0.93300	4	0.46858	4	4.09	2
30	1865	33205	80.99	−1.03828	5	0.57386	5	5.01	0.2
31	1314	34519	84.19	−1.12644	6	0.66202	6	5.78	−4
32	1422	35941	87.66	−1.24261	7	0.77819	7	6.79	−3

* Total number estimated from values for other ages in tables and checked by computation.

† The average annual values for lolog difference in this table was taken as one-twelfth of the difference between ages 18 and 30, or ±0.114627.

‡ This age group reached age 20 in year 1920, the year of the return of soldiers from the war. The lower ages show the effect of the war, and the upper ages the effects of the period of prosperity and depression.

Next the logs of these percentages of development were found and plotted (Figure 4). The uniformity of the rate of log development is evidenced by the straightness of the resulting curves.

In the table, age 25 was chosen as a reference point, and the log and time differences from the values at this point were computed. Then the ratios of these differences from the reference point to the average yearly (or five year) differ-

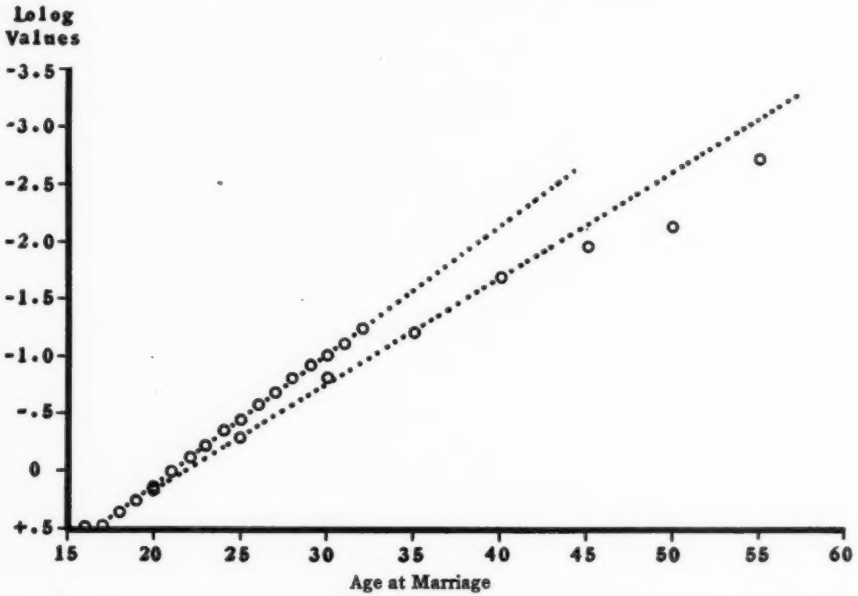

FIG. 4. Attainment of marital status. Graphs of the lolog values from columns 5 and 5a, Table 1. The lolog values of the percentages of development of *both* single-cycle growth curves form straight lines within the limits of error of the data.

The figure presents graphically the factual basis for the law of maturation: namely, that for *all* single-cycle maturation curves the *increases* in the lolog values of the percentages of development measured from a given point of reference, are *directly proportional* to the *increases* in time measured from the same point of reference.

ence were found, compared, and the percentages of error between the lolog ratios and the time ratios computed. (Column 10, 10a) The results indicate that the increase in lolog values from the given point of reference are proportional to the increases in the times to within about seven per cent for the heterogeneous group, and about three per cent for the single age group.

The comparison of these two series of results illustrates the important fact that as constancy of conditions increases the percentages of error grow smaller. The formula is believed to represent the relationships that would obtain if absolutely constant conditions could be maintained throughout the cycle.

For instance, the equations for the straight lines of best fit for the curves shown in Figure 4 are:

$$\text{Small group: } \text{Lolog}\left(\frac{y}{242}\right)=2.01328-0.092466t$$

$$\text{Large group: } \text{Lolog}\left(\frac{y}{41,000}\right)=2.40126-0.114627t$$

These equations cleared of logarithms become:

Small group: $y=242(0.7852\times10^{-102})^{0.80823^t}$

Large group: $y=41,000(0.12064\times10^{-250})^{0.789^t}$

Both sets of equations seem formidable to one who is not skilled in mathematics. However, by the use of a special symbol,][ ], signifying that the expression within the brackets is to be cleared of logarithms before it is combined with the other elements in the formula, the equations may be written in the simple form:

Small group: $y=242][2.01328-0.092466t]$

Large group: $y=41,000][2.40126-0.114627t]$

In this form computations may be made by simple arithmetic, the change of the quantities within the brackets to percentages of development being made by an appropriate table. In this article all further equations will be expressed in this simple form.

The mid-differences between the values for the different ages computed by these equations, and the actual observed values given in columns 2 and 2a are, for the small group, two men (base 242), and for the large group 256 men (base 41,000). Very many curves are in the possession of the writer in which the errors of prediction fall well within a two per cent difference from actual values. (See Table 2 page 168.)

GENERALIZATION

Two swallows do not make a summer, nor do two curves establish a law. But when curves from some thirty different but similar groups of men and women, involving more than a million and a half persons, all conform to one pattern (Figure 5, 6), and when, further, hundreds of similar single-cycle curves, both group and individual, from many different types of fields of biological research, exhibit the same characteristics, the time comes when it is possible to generalize and claim justly that single-cycle growth in any field follows the distinctive pattern represented by the formula above.

In somewhat similar fashion, equations were computed* for the four types of multi-cycled curves shown in Figure 1, assuming them to be compounded from two or three cycles as their appearances suggest. It was found that the various portions of these complex curves may all be described quite precisely in terms of the formula, or law. (Table 2.) That is, many complex curves are merely the resultants of compounding two or more cycles of growth.

* The computations for all results presented in this article are on file with the secretary. A copy will be sent to anyone who sends 12¢ in stamps with his request. Address S. A. Courtis, School of Education, University of Michigan, Ann Arbor, Michigan.

TABLE 2

EQUATIONS FOR THE COMPLEX GROWTH CURVES IN FIGURE 1

Curve	Equation	Mean Deviation
A	$y=15][-0.015361t-0.01776]+27][-0.058168+1.88868]$	±0.5 cm.
B	$y=82][-0.61109t-0.69308]+19][-0.84756t+1.12258]$ $+57][-0.14828t+0.60224]+31][0.34550t+4.48586]$	±0.9 cm.
C	$y=145][-0.041794t+3.23163]+50][-0.108765t+15.02223]$	±0.6 months
D	$y=\ 24{,}000][-0.023092(t-1800)+0.54886]$ $+130{,}000][-0.027193(t-1800)+1.31048]$ $+400{,}000][-0.030709(t-1800)+2.63039]$ $+3{,}500{,}000][-0.029854(t-1800)+3.48914]$	±6% excluding depression years

SPECIAL COMMENTS

Curve B. The boy was measured in July and in January. Seasonal variation in the amount of growth is evident in the errors. However, the average error includes these seasonal changes. Half the errors were 0.6 cms. or less. Three of the largest errors, 4.3, 2.1, and 3.3 cms. occur at ages 2.5, 3.0, and 3.5 years and might easily have been occasioned by sickness.

The formula is a statistical formula of fit only, as the size at conception, by this formula, would be 16.6 cms., a ridiculous figure. The reason is probably that there are at least two prenatal cycles and the formula only provides for one.

Curve D. Detroit was established as a fort and trading post in 1703 by the French. The English took possession in 1760 and maintained the settlement as a fort. It was turned over to the United States in 1796 but British influence was not completely eliminated until after the War of 1812. The early records of population are not very reliable. The first United States Census was in 1820 and the population of Detroit as an American city is taken from 1819.

The error at each year was computed by dividing the difference (between the population as computed by the formula and the actual population) by the actual population. The A.D. of 5% is the mean of 67 population estimates, omitting the ten depression years: 1830 and 1834, following the War of 1812; 1860, 1864, and 1870, the Civil War depression; and 1930, 1932, 1934, 1935, 1936, the World War depression years. The A.D. for these years was 38%. Three other large errors occurred between 1840 and 1850.

The data are quite unreliable. As shown by the dots in the figure, the estimates of the United States Census, the Water Board, the Board of Education, and the Polk Directory for any one year sometimes vary by as much as 50,000 from high to low. Some estimates include the city proper, others the metropolitan area. The curve follows the general trend. Hence the relatively large error of approximately 6%, about twice the average error of biologic curves.

EXPLANATIONS

Empirical determinations of law are not completely satisfying; one desires also a rational explanation in terms of cause and effect. Fortunately in this case, such an explanation is known and will be given in detail in a later paper. Moreover, the relationships between factors postulated in the explanation are subject to experimental proof.* For the present, however, the reader is asked to accept only the empirical facts, and to consider the implications of the generalization if ultimately it is accepted by scientists generally.

* The difference between the statistical and the experimental approach is fundamental. On the one hand the statistician prides himself on his ability to twist a curve *through any data* by adding mathematical constants and terms to his formula. Therefore he deals with data as he finds them. On the other hand an experimentalist seeks constantly to reduce the number of constants in his formula through

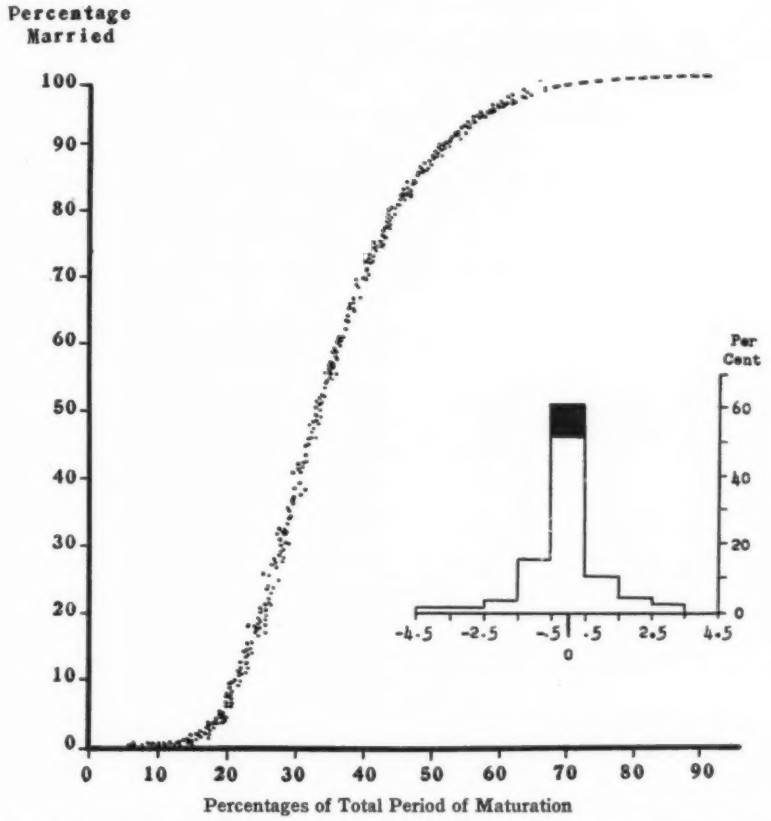

FIG. 5. Attainment of Married State. Thirty group maturation curves—fifteen for men and fifteen for women—superimposed on a common base.

Each point represents the percentage of a group of approximatly 40,000 Australian men (or women) born the same year who were married at the time the observation was made. Data taken from government reports, 1908–1932. Total number of individuals about a million and a quarter. Total observations, 540. Sixty-two per cent of observations deviate from values computed by formula 0.5 of one per cent or less, as shown in the small diagram.

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control of the elemental factors, and to make each constant symbolic of an objectively identifiable factor.

The number of formulae used to describe growth curves is legion. A few of the more important of the statistical formulae are:

$y = a + bt + ct^2$	A parabola
$\log p = k_0 + k_1 \cdot \log T - k_2 \cdot \log^2 T$	Backman
$\log\left(\frac{x}{A - x}\right) = K(t - t_1)$	Robertson
$y = A(1 - e^{-kx})$	Mitscherlich
$y = A(1 - R^x)$	Spillman
$y = d + \frac{k}{1 + e^{a_1x_1 + a_2x_2 + a_3x_3 \cdots a_nx_n}}$	Pearl

The ideal single-cycle maturation curve is a skew curve (Figure 7). The point of inflection occurs at e^{-1} and, in time, at approximately one-third the total period of growth. The curve is asymptotic both to the zero development axis and to the 100% line, but for practical purposes the starting and stopping points may, without serious error, be taken as those points at which change in magnitude *is no longer measurable.*

The skewness of the curve is most easily observed when growth is expressed as a distribution of amounts of growth made in the successive unit of time (Table 1, columns 2 and 2a). Much use has been made in statistical analyses

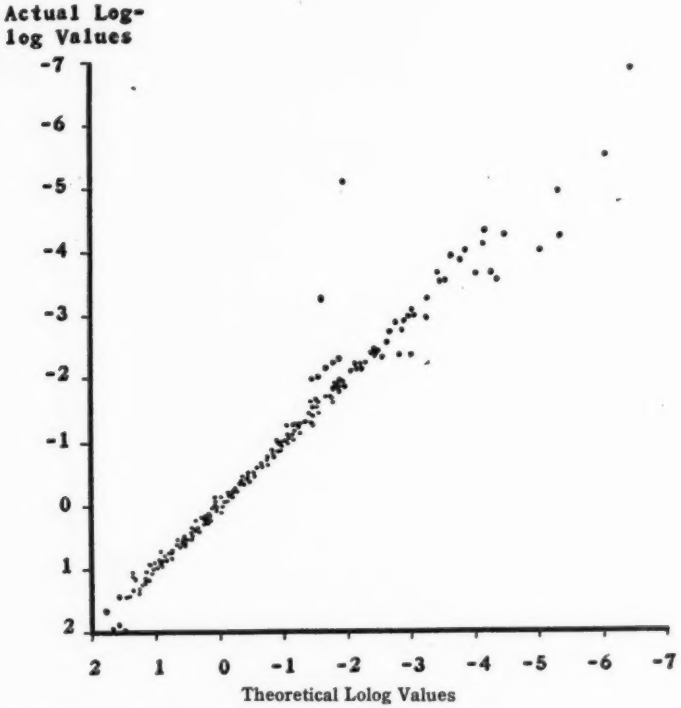

FIG. 6. Derivation of Formula Describing Maturation Curves.

A. *Factual Basis*—The logs of the percentages of maturation form a straight line. Actual Napierian lolog values of percentages of maturation are plotted against theoretical values; twenty curves from five different biologic fields are represented, 204 observations in all.

B. *Mathematical Representation of Relationship*

General equation of straight line	$y=mx+b$
Equation of line of best fit above	$\text{lolog } y=mx+b$
Take antilog	$\log y=e^{(mx+b)}$
	$=e^{mx}\cdot e^{b}$
Substitute a for m, t for x and c for e^{b}	$\log y=ce^{at}$
Take antilog	$y=e^{ce^{at}}$
Substitute i for (e^{c}), r for (e^{a})	$y=i^{r^{t}}$

of biological data of the concept of "normal distribution" but in dealing with growth data the use of statistical methods based on an *assumed* normal distribution is invalid because such distributions are skew. Maturation units based on the skew growth curve eliminate such errors.

The form of the curve has led certain biologists to assume that there are "growth-promoting" substances and "growth-retarding" substances whose operation "causes" the growth curve to assume its peculiar form. While experiments prove that there are growth-promoting and growth-retarding substances (Leonian '35), it does not follow that one type of substance acts at one

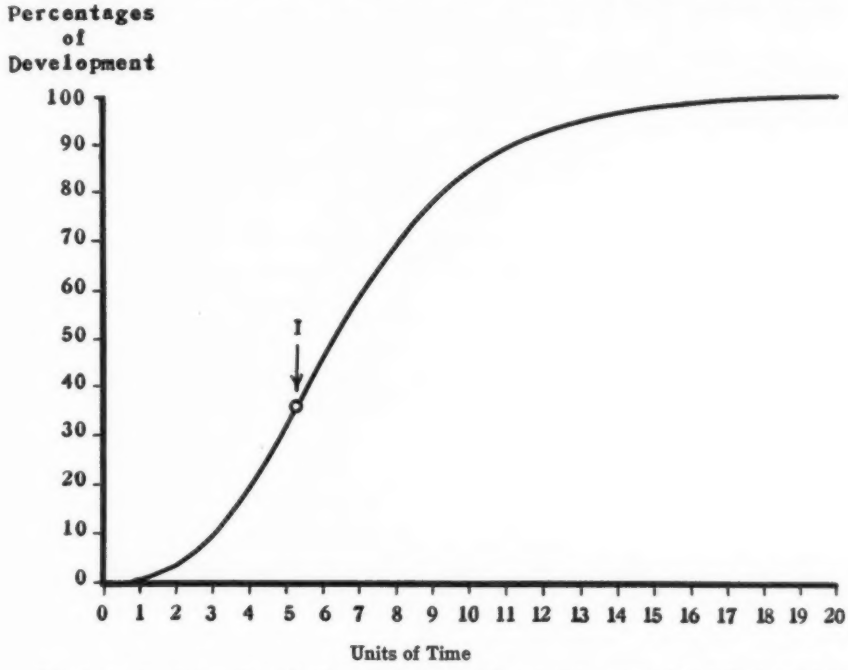

FIG. 7. A computed, mathematically perfect, single-cycle maturation curve. The inflection point I, occurs at 36.8% of development or at about one-third of the total observable period of maturation. The curve is markedly skew.

portion of the curve and the other type at a later portion of the curve. Cycle as here defined *postulates absolutely constant conditions* throughout the whole growth period. The peculiar form of the curve can be explained without the slightest references to such assumptions of change in substances acting.

OBJECTIONS

The general reactions of biologists to attempts to limit study of growth curves to description only are the conventional responses: "Not true," "Unimportant if true," and "The mathematics involved is too complicated for practical use." Each of these objections will be discussed in turn.

DISTORTED CURVES

The truth or falsity of any statement in science is not to be established by dogmatic expressions of opinion by authorities no matter how great the reputations of the persons expressing opinions may be. In this case, the empirical facts about single cycle growth curves have been recognized more or less clearly by many observers and may be verified independently by anyone who cares to make the necessary measurements and computations. There are of course many so-called growth curves which are not single-cycle maturation curves at all. There are also many developmental curves which do not conform to the pattern above, but in all such cases variations from the conditions set up as standard by definition* will be found to offer adequate explanation of the departures from the standard pattern. Thus, once the definition of cycle has been accepted, departures from the normal pattern become of diagnostic significance.

Therefore the biologist who dismisses lightly the ideas presented above because they do not "*agree with his experiences*" is making a serious mistake. He should investigate "maturation under *constant* conditions" before reaching a conclusion as to truth or falsity of the ideas presented. For in science the key to the analysis of complex situations has always been the securing of precise and complete knowledge of the simple and the regular.

RELATION TO QUALITATIVE ANALYSIS

Biologists who are trying to discover the nature of growth experimentally seldom regard precise mathematical description as of much significance. For instance Hammet says ('35 p. 59):

> Absence of consideration for exponential analysis of growth curves . . . has been purposeful—interest here (i.e. in his monograph) is in the quality not the quantity of the increment—indeed singularly naive . . . seems the belief that because increase on time charts a certain course, conclusions must be made that total growth is a bimolecular, a monomolecular or even an autocatalyzed monomolecular reaction.

The writer interprets Hammett's comments to mean that because his specific interest is in quality, he believes there is little or no likelihood that anything of value can be learned *about quality* from a study of quantity. In other words, he regards mathematical description as unimportant for his purposes.

Such a statement of belief raises a crucial question, the relation of the qualita-

* Even the simplest one-celled organisms that can be studied may be analyzed into many tissues, structures, parts, etc., while most plants and animals are organized populations of hundreds, thousands, or even millions of cells. These various cells or parts may be called "elements" and are found to exhibit individual differences. Under standard conditions the distribution of these individual differences will be "normal." A change in the form of this distribution of differences will be reflected in the form of the growth curve. Hence the normality of the distribution of individual differences among the nature elements in the organism must be included as one of the conditions necessary to produce the standard pattern of growth. The conditions selected as standard are those most frequently found in nature. Strictly speaking, however, the pattern of growth during a single cycle will conform to the standard pattern only under "standard" conditions.

tive to the quantitative in science. On the one hand no one can deny that all new discoveries are made qualitatively *first*, but on the other hand it is equally true that no qualitative discovery is ever brought under effective control until it has been formulated in mathematical terms.

On this point Pearson (1892 pp. 395, 398) says:

If we look upon biology as a conceptual description of organic phenomena, then nearly all the statements we have made with regard to physics will serve as cannons for determining the validity of biological ideas. In particular any biological concept will be scientifically valid if it enables us to briefly summarize without internal contradiction our perceptual experiences. But the moment the biologist goes a step farther and asserts on the ground of the validity of his concept that it is a reality of the phenomenal world, although no perceptual equivalent has yet been found for it, then he at once passes from the solid ground of science to the quicksands of metaphysics.

It is this transition from science as conceptual description of the sequence of sense impressions to metaphysics as a discussion of the imperceptible substrata of sense impressions which mars biological as well as physical literature.

Hammett, of course, is making a precisely similar protest when he calls conclusions drawn from mathematical description "naive," but one has only to read the remainder of his 306 theses to realize how futile it is to pile one qualitative hypothesis upon another when none of them rests on a quantitative foundation. The process of mathematical description of cycles of growth *is* relatively unimportant in itself. What makes it significant is that, once mathematical description is possible, the effect upon the growth curve of *any single qualitative difference in conditions*—such as war in the illustration above—can also be determined precisely by measuring the amount of departure from the normal cyclic growth pattern produced by the factor being varied. In later papers specific illustrations in many different fields will be presented to demonstrate the way in which the concept of cycle as herein defined may be used *as a tool* to establish the truth or falsity of those very qualitative aspects of the nature of growth in which Hammett and other biologists are most interested.

MATHEMATICAL DIFFICULTIES

As for mathematical difficulties, the cycle concept simplifies rather than increases them. While it is true that for pioneering in mathematical description, a working knowledge of higher mathematics is essential, once the key to mysteries has been discovered, actual procedures can be reduced to simple arithmetic. Using maturation units and the new techniques that have been developed the computation of growth equations can be carried on by any clerk with a common school education, who can follow directions. The most difficult of the routine computations can be performed easily by those able to use logarithms.

THE NEED FOR CRITERIA

A really important objection to the cycle concept is implied in the question frequently asked: "How can one tell with certainty whether he is dealing with

a curve of two or more cycles, or merely with trivial variations in the constancy of conditions, or in accuracy of measurement? The answer is that there are definite and measurable criteria for determining the presence of two or more cycles. Discussion of these criteria will be taken up in the next paper in this series.

CONCLUSIONS

From all the evidence available it seems reasonable

1. To define a cycle of growth as a period of specific maturation during which all the elements and forces acting are *constant* within allowable errors of control and measurement.

2. To draw conclusions with respect to the pattern of growth from a study of relatively pure cycles only.

3. To consider departures from the standard pattern of cyclic growth as indices of the effects of the disturbing factors.

Once these conclusions are accepted, the mathematical description of growth curves becomes a powerful diagnostic tool for the study and precise measurement of the effect of factors potent to promote, retard, or accelerate growth. In succeeding papers in this series, the writer hopes to prove that as a result of the clarification of the growth cycle situation by precise definition, the road is open for a new and promising type of attack upon many of the persistent problems of biology.

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